REPORTS

OF THE

SLEEPING SICKNESS COMMISSION

OF THE

ROYAL SOCIETY.

No. X.

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[*Reprinted from the* Proceedings of the Royal Society,
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29. FURTHER RESULTS OF THE EXPERIMENTAL TREATMENT OF TRYPANOSOMIASIS: BEING A PROGRESS REPORT TO A COMMITTEE OF THE ROYAL SOCIETY.

By H. G. Plimmer, F.L.S., and H. R. Bateman, Captain R.A.M.C.

(Communicated by J. Rose Bradford, M.D., F.R.S. Received August 25th, 1908.)

The following results are a continuation of the work of which summaries have already appeared in the "Proceedings of the Royal Society."*

The experiments have been carried out with the same strains of Nagana and Surra as were used before.

A.—*Condition of the Animals living at the Date of the Completion of the Tables in the last Paper.*

Table I.—Nagana Rats treated with Atoxyl and Succinimide of Mercury.

(Average duration of untreated disease, 5·5 days.)

No. 4 died on the 307th day after inoculation.
„ 7 „ 365th „
„ 10 „ 249th „
„ 15 „ 188th „
„ 21 „ 63rd „

Of these, No. 15, which was apparently cured, was used on the 147th day after inoculation for re-inoculation, with the view of ascertaining if any immunity had been conferred. This was found not to be the case.†

None of the above died with any of the signs of Nagana. Of the 21 rats tabulated,‡ only one died from trypanosomiasis, and this one was probably atoxyl-proof.

Table III.—Surra Rats treated with Atoxyl and Mercury Sozoiodol.

No. 5 died on the 286th day after inoculation from bronchopneumonia.

Table VI.—Surra Rats treated with Atoxyl and Iodopin.

No. 9 died on the 221st day after inoculation from worms.

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* B, vol. 79, 1907, pp. 505–516, and B, vol. 80, 1908, pp. 1–12.
† *Vide* 'Roy. Soc. Proc.,' B, vol. 80, p. 10.
‡ 'Roy. Soc. Proc.,' B, vol. 79, p. 510.

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Rats treated with Atoxyl and Liq. Hydrarg. Perchlor.

The rat still living at the date of the last paper died on the 187th day after inoculation from broncho-pneumonia.

Rats treated with Sodium Antimonyl Tartrate. (Table on pp. 8—9 of the last Paper.) Results to August* 20.

No. 7 *is living and well* 354 *days after inoculation.*
„ 8 died on the 194th day „
„ 9 „ 323rd „ „
„ 10 „ 145th „ „
„ 14 „ 48th „ „
„ 15 „ 67th „ „
„ 16 „ 227th „ „
„ 17 „ 227th „ „
„ 19 „ 161st „ „
„ 20 „ 49th „ „
„ 21 „ 210th „ „
„ 23 „ 104th „ „
„ 25 „ 134th „ „
„ 26 „ 134th „ „
„ 27 „ 33rd „ „
„ 28 „ 34th „ „
„ 29 „ 57th „ „
„ 30 „ 28th „ „
„ 31 „ 147th „ „
„ 32 *is living and well* 346 *days after inoculation.*
„ 33 died on the 228th day „
„ 34 „ 188th „ „
„ 35 *is living and well* 346 *days after inoculation.*
„ 36 died on the 228th day „
„ 38 „ 173rd „ „

It will be noticed that nine of the above rats lived for over 200 days, and nine others considerably over 100 days. Of those which have died only four have had recurrences (two had one, and two had three recurrences); none of them died with any symptoms of trypanosomiasis, and in none were trypanosomes found after death. An emulsion of the liver and of the bone-marrow of Nos. 9, 16, 17, 21, and 25 was injected into other rats with negative results in every case.

Rats treated with equal parts of 1 *per cent. Solutions of Sodium Antimonyl Tartrate and Sodium Arsenyl Tartrate.*

The five rats living at the date of the last paper have died:—

No. 1 died on the 199th day after inoculation.
„ 2 „ 26th „
„ 3 „ 43rd „
„ 4 „ 59th „
„ 5 „ 40th „

As was anticipated from the earlier experiments, this treatment has no advantages over that with antimony alone.

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* 'Roy. Soc. Proc.,' B, vol. 80.

B.—*Further Experiments.*

Potassium Antimonate.

This was tried in doses up to 7 minims of a 1 per cent. solution. In this dose it was poisonous to rats, and it did not kill the trypanosomes.

Atoxyl and Sodium Antimonyl Tartrate.

This was given in doses of 5—7 minims of a 5 per cent. solution of atoxyl and of a 1 per cent. solution of sodium antimonyl tartrate. Three Nagana rats lived respectively 23, 43, and 41 days; they all had recurrences (the last had five) and died with living trypanosomes in the blood.

Pushing the drug does not have any good effect (*see* next table); the trypanosomes are not driven out more quickly, or more effectually; recurrences are more common, and inflammatory intestinal lesions were present in nearly every case. Two rats died with living trypanosomes in the blood: these two had become antimony-proof, as the later doses did not remove the trypanosomes from the blood, nor make any difference in their number. This strain did not maintain this quality for long, as after the second sub-inoculation from these rats the trypanosomes seemed to have become normal in their behaviour towards antimony. The strain was lost at this point.

Nagana Rats treated with Large Doses of Sodium Antimonyl Tartrate.

No.	Weight in Grammes.	Total Quantity of 1 per cent. sod. ant. tart. given, in c.c.	Recurrences.	Results.
1	200	5·75	1	Died on 30th day with intestinal lesions.
2	125	5·0	0	Died on 34th day with very marked intestinal lesions.
3	100	4·75	0	Died on 24th day: intestines necrotic.
4	100	4·0	0	Died on 103th day from pneumonia.
5	110	4·9	1	Died on 43rd day with inflamed intestines.
6	110	6·5	2	Died on 38th day with necrotic intestines.
7	175	8·5	2	Died on 40th day: trypanosomes in blood at death; paralysed.
8	110	9·0	2	Died on 45th day in similar condition to No. 7.
9	150	5·0	4	Died on 73rd day from gangrene of tail.
10	175	5·25	1	Died on 64th day from pneumonia.

Rats treated with Sodium Antimonyl Tartrate after Inoculation with Atoxyl-proof Trypanosomes.

The rats in the following table were all treated firstly with atoxyl to make sure that they were atoxyl-proof, and the treatment with antimony was then begun, when the trypanosomes were in large numbers in the blood.

—	No.	Weight in Grammes.	Total Quantity of 1 per cent. sod. ant. tart. given, in c.c.	Recurrences.	Results.
Nagana.	1	100	3·0	1	Died on 29th day with trypanosomes in blood.
	2	100	1·0	0	Died on 11th day from nephritis.
	3	110	3·0	1	Died on 54th day.
	4	110	3·0	1	Died on 20th day with trypanosomes in blood.
	5	175	3·0	0	Died on 117th day from broncho-pneumonia.
Surra	6	100	3·0	0	Died on 27th day from septicæmia.
	7	145	5·75	1	Died on 41st day from pneumonia.
	8	140	4·0	1	Died on 38th day with trypanosomes in blood.

It will be seen that the atoxyl-proof strains of trypanosomes are less influenced by antimony than are the ordinary variety, as three of the above rats had living trypanosomes in the blood at death, and seven of them died at a very early date.

Rats treated with Antimony (Metal) and Sodium Antimonyl Tartrate suspended in a Fatty Medium.

Sodium antimonyl tartrate, when injected in watery solution into man produces very severe pain and inflammation in the neighbourhood of the injection, with more or less local necrosis. In order to make the use of antimony practicable in the form of injection, a series of experiments was undertaken, using various other media than water for solution or suspension of the antimony salt. Lanolin, olive oil, and sesamum oil were tried, but the results were not good. Finally, the medium Colonel Lambkin devised,* consisting of palmitin and antiseptics, which is used very largely for the intramuscular injection of calomel and mercury in syphilis, was tried, with the results which are set forth in the tables below.

One great advantage of these preparations is that they can be used upon man with far less difficulties and after-consequences than the watery solutions, which seem to be impracticable; this is of importance should antimony be found of use in human trypanosomiasis.

Major Ward, R.A.M.C., has used both the forms mentioned above on men for other purposes, and he has very kindly placed his notes at our disposal. In one of his cases four doses of ½ grain of sodium antimonyl tartrate, suspended in Colonel Lambkin's medium, were given intramuscularly into the buttock, the intervals between the doses being 3, 4, and 3 days: the doses were then increased to 1 grain, and seven doses of this strength were given, the intervals being 4, 3, 2, 4, 2, and 13 days. In all, this patient had 9 grains of the salt. Major Ward says that "the injections caused a certain amount of tenderness and discomfort at the seat of injection," but that is very different to the effects noticed after the injection of the watery solution.

Major Ward also treated two patients with antimony itself in a state of extremely fine division suspended in Colonel Lambkin's medium; they were each given one dose of 1 grain, and 11 days afterwards ½ grain. This form caused both pain and discomfort, and also a general increase in the size of the buttock, into which the injection was made, but this subsided without suppuration. This form would appear, however, to be much the more powerful of the two, as the effects obtained from the 1½ grains of the metal were as good and as lasting as those observed in the case in which 9 grains of sodium antimonyl tartrate were given.

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* 'Journ. Roy. Army Med. Corps,' 1906.

The following tables show the results obtained in rats with sodium antimonyl tartrate and antimony prepared as mentioned above:—

Sodium Antimonyl Tartrate, 5 per cent., in Colonel Lambkin's Medium.

—	No.	Weight in Grammes.	No. of Doses given.	Total Quantity given, in Minims.	Recurrences.	Results to August 20.
Nagana.	1	100	4	18	2	Died on 53rd day: bad itch.
	2	100	2	6	1	Died on 16th day with intestinal lesions.
	3	115	1	2	0	Died on 113th day of pneumonia.
	4	150	1	2	0	Died on 19th day of pneumonia.
	5	110	5	17	1	Died on 27th day: abscess.
	6	125	3	13	0	Died on 26th day of pneumonia.
	7	150	3	13	0	Died on 89th day: itch.
	8	175	1	3	1	Died on 24th day: abscess.
Surra	9	200	3	17	1	Died on 40th day of injury.
	10	225	3	13	2	Died on 28th day with intestinal lesions.
	11	115	2	6	1	Died on 22nd day of pneumonia.
	12	220	11	33	7	Died on 66th day with acute intestinal lesions.
	13	125	1	3	0	*Living and well* 154 *days after inoculation.*

None of the above have died from trypanosomiasis. In rats the intra-muscular injection of even a few minims is difficult, and if any of the material be left under the skin a slough forms, which takes a long time to heal. It will be noticed that No. 3 had only one dose: the rat lived 113 days and died of pneumonia; inoculations made from its organs were negative. No. 13, which is still living (154 days), has also had only one dose.

From the following table it will be seen that the administration of the metal itself has a considerable effect on the trypanosomes: it has a distinctly better effect on Surra than upon Nagana, four Surra rats out of 16 being still alive, and four others having lived for a long time. In none of the Surra rats were trypanosomes found at death, whereas in three of the Nagana rats they were present. The metal is much more irritating than the tartrate, but the effect is in most cases more prolonged; this is probably due to the fact that the absorption of the metal is much slower. Further, the smaller doses would appear to be the most efficient.

Antimony, in a state of very fine division, 5 per cent., in Colonel Lambkin's Medium.

—	No.	Weight in Grammes.	No. of Doses given.	Total Quantity given, in Minims.	Recurrences.	Results to August 20.
Nagana.	1	100	3	12	3	Died on 61st day : abscess.
	2	200	3	11	2	Died on 29th day with trypanosomes in blood.
	3	100	3	9	1	Died on 34th day with intestinal lesions.
	4	110	3	12	1	Died on 15th day with trypanosomes in blood.
	5	100	4	14	1	Died on 27th day with intestinal lesions.
	6	150	4	15	2	Died on 26th day with intestinal lesions.
	7	150	6	23	3	Died on 33rd day with trypanosomes in blood.
	8	300	3	13	4	Died on 51st day from bronchopneumonia.
	9	300	2	8	2	Died on 36th day from bronchopneumonia.
	10	200	2	8	0	*Living and well* 204 *days after inoculation.*
	11	300	2	8	1	Died on 29th day : abscess.
	12	250	2	8	1	Died on 39th day from bronchopneumonia.
	13	250	2	8	7	Died on 92nd day from pneumonia.
	14	300	1	5	0	Died on 32nd day : fatty liver.
	15	300	1	5	0	Died on 30th day.
	16	150	1	3	0	Died on 74th day from pneumonia.
Surra	17	110	1	5	0	*Living and well* 230 *days after inoculation.*
	18	125	4	17	4	Died on 146th day : one recurrence took place after an interval of 12 weeks.
	19	250	4	35	3	Died on 48th day from bronchopneumonia.
	20	225	1	4	0	Died on 39th day from bronchopneumonia.
	21	100	1	4	0	Died on 44th day : itch.
	22	100	3	10	1	Died on 44th day : itch.
	23	150	2	9	1	Died on 216th day of pneumonia.
	24	100	3	12	3	Died on 94th day : abscess. One recurrence took place after an interval of 46 days.
	25	100	2	9	0	*Living and well* 253 *days after inoculation.*
	26	150	2	9	0	Died on 29th day from pneumonia.
	27	125	1	3	0	*Living and well* 226 *days after inoculation.*
	28	100	1	3	0	Died on 91st day : itch.
	29	100	1	3	0	*Living and well* 226 *days after inoculation.*
	30	125	1	4	0	Died on 27th day from pneumonia.
	31	100	1	4	0	Died on 27th day from pneumonia.
	32	150	1	4	0	Died on 41st day : itch.

Experiments in which Antimony and Sodium Antimonyl Tartrate were given before Inoculation, in order to Test their Effects upon the Development of the Disease.

In these experiments the substances were given suspended in Colonel Lambkin's medium, and it will be noticed that the metal is far more effective in delaying the appearance of the trypanosomes in the blood than is the salt: this is probably due to the slower elimination of the metal. This method, if the doses were repeated, might be of some practical value in getting animals safely across dangerous tracts of country.

Four Rats were given 5 minims of 5 per cent. Antimony (metal) Cream on December 19. In Nagana the trypanosomes usually appear in the blood on the second or third day after inoculation.

No.	Inoculated with Nagana on—	Trypanosomes appeared in the blood on—
1	December 20 ...	December 31, the 11th day.
2	„ 21 ...	January 2, the 10th day.
3	„ 23 ...	December 31, the 8th day.
4	„ 24 ...	„ 30, the 6th day.

Four Rats were given 3 minims of 5 per cent. Sodium Antimonyl Tartrate Cream on December 19.

No.	Inoculated with Nagana on—	Trypanosomes appeared in the blood on—
1	December 20 ...	December 25, the 5th day.
2	„ 21 ...	„ 24, the 3rd day.
3	„ 23 ...	„ 26, the 3rd day.
4	„ 24 ...	„ 27, the 3rd day.

Rats treated with Lithium Antimonyl Tartrate.

There are differences in the effects produced by the potassium, sodium, and lithium antimonyl tartrates, if given under similar conditions and dosage. The commercial potassium salt is very impure. The pure sodium and lithium salts which we have used have been prepared for us by Dr. R. H. Aders Plimmer, of University College. The sodium salt contains roughly about 2 per cent. more antimony than the potassium salt, and the lithium salt contains about 2 per cent. more antimony than the sodium; but the doses of the lithium salt have to be much smaller than the corresponding doses of the sodium salt. For instance, 0·5 c.c. of a 1 per cent. solution of the lithium salt is fatal to a rat of 125 grammes, and 0·39 c.c. is fatal to a rat of 80 grammes. When the watery solution is injected intramuscularly, it has not caused necrosis of the tissues in rats, but subcutaneously it has occasionally done so. We have found that the best strength of solution for rats is 0·25 per cent., and of this 0·5 c.c. has been given for a dose.

The following table shows the effects of this dosage:—

—	No.	Total Quantity in c.c. given of 0·25 per cent. solution.	Recurrences.	Results to August 20.
Nagana.	1	7	2	Died of disease on 47th day.
	2	7	1	Killed on 47th day, owing to abscess. Emulsion of organs injected into another rat gave negative result.
	3	4·5	1	Allowed to die on 34th day, the date of recurrence.
	4	4·5	0	*Alive and well* 134 *days after inoculation.*
	5	4	0	*Alive and well* 125 *days after inoculation.*
Surra	6	4	0	*Alive and well* 134 *days after inoculation.*
	7	4	0	Died on 42nd day of broncho-pneumonia: no evidence of trypanosomes.
	8	4	0	*Alive and well* 134 *days after inoculation.*
	9	5	2	Died of disease on 47th day.
	10	4·5	0	*Alive and well* 125 *days after inoculation.*
	11	3·5	2	Died of disease on 18th day: itch.
	12	3	1	Allowed to die: relapse not treated.

From the above it will be seen that five out of these 12 rats are alive and well at periods varying from 125 to 134 days. This salt is much more soluble than either the potassium or sodium compound, which may, perhaps, as well as its greater antimony content, account for its greater effectiveness. It is, however, more irritating subcutaneously in watery solution.

Experiments with Antimony upon Dogs.

In order to see what the effects of antimony would be on the larger and more important animals when suffering from trypanosomiasis, a series of experiments on dogs has been begun. The trypanosome used was that of Surra, which kills dogs of about 20 lbs. in weight in approximately 14 days, as this is the trypanosome which is of practical importance with regard to dogs.

Dr. MacConkey, of the Lister Institute, kindly performed some initial experiments for us. He made some experiments with a 20 per cent. suspension of sodium antimony tartrate in Colonel Lambkin's medium, in order to find the minimum lethal dose, and he found that 0·5 c.c. of this 20 per cent. cream per 10 lbs. of body weight was probably about the full dose. But he also found that, for practical purposes, this 20 per cent. cream was much too strong, as it caused sloughing in every case; indeed, dogs do not seem to bear these suspensions as well as they do solutions. Dr. MacConkey kept one dog alive till the 40th day, and one until the 30th, but both had many relapses.

Profiting by the experience thus gained, we have made further experiments using much smaller doses, with satisfactory results. But we have found, since trying the lithium antimonyl tartrate that this acts more effectually and with less irritation than the creams, whether of metal or salt. All the animals tabulated below are in good condition and are gaining in weight.

(Average length of untreated disease, 14 days.)

No.	Weight, in lbs.	Total Quantities given, in Minims.	Recurrences.	Results to August 20.
1	22	Antimony cream, 5 per cent., 60 m. Sod. ant. tart., 2 per cent., 40 m.	2	No trypanosomes present since July 13 ; is alive and well on the 62nd day after inoculation.
2	22	Sod. ant. tart., 2 per cent., 80 m. Lith. ant. tart., 2 per cent., 55 m.	2	Had 9 pups during treatment ; there were 42 days between the two recurrences ; is alive and well on the 62nd day after inoculation.
3	37½	Sod. ant. tart. cream, 5 per cent., 80 m. Sod. ant. tart., 2 per cent., 40 m. Lith. ant. tart., 2 per cent., 90 m.	6	Incorrect dosage seems a probable cause of these relapses ; is alive and well on the 62nd day after inoculation.
4	16	Sod. ant. tart. cream, 5 per cent., 40 m. Sod. ant. tart., 2 per cent., 20 m. Lith. ant. tart., 2 per cent., 40 m.	3	Is alive and well on the 53rd day after inoculation.
5	13½	Sod. ant. tart. cream, 5 per cent., 35 m. Lith. ant. tart., 2 per cent., 47 m.	3	Is alive and well on the 53rd day after inoculation.

The second and third substances mentioned in Column 3 were given at the recurrences. That these dogs are all alive and well encourages us to hope that, as we get a better knowledge of the dosage required, the recurrences may be less frequent.

Experiments made with Rats treated with Antimony, in order to find out in what Organs the Trypanosomes are latent.

The following initial experiments were made in order to find out where the trypanosomes rest during the period in which the peripheral blood is free from them, after treatment with antimony. Further experiments are being carried on for the purpose of ascertaining in what forms they are present in the organs of treated animals.

The rats were inoculated with Nagana, which is less affected by antimony than Surra, and were all treated with four doses of sodium antimony tartrate. The rats were killed at various intervals, and the organs selected (the liver and bone-marrow) were made into an emulsion with a minimum quantity of 0·75 per cent. salt-solution, and injected into other rats in doses of 1 c.c. ; the same dose of blood from the heart was also given.

In the following table the signs + and − are used respectively to denote a positive or negative result.

No.	Number of Days after treatment upon which Rats were killed.	Blood.	Liver.	Marrow.
1	7	−	−	−
2	10	−	−	−
3	12	−	−	−
4	14	−	−	−
5	2	−	+	+
6	4	−	+	−
7	3	−	+	+
8	12	−	−	+
9	14	−	−	+
10	16	−	−	+
11	20	−	−	+

From this table it would appear that the bone-marrow is the place where the trypanosomes can live longest, and that the liver is also a place where they can find protection. This is borne out by some experiments we have made upon trypanosomiasis in birds, in which cultivations of trypanosomes can often be made from the bone-marrow when they cannot be made either from the organs or the blood. The doses given to the above rats were rather under those which we should judge to be curative, but in four cases the results were entirely negative.

[*Reprinted from the* Proceedings of the Royal Society, B. Vol. 81.]

30. A TRYPANOSOME FROM ZANZIBAR.

By Colonel Sir David Bruce, C.B., M.B., F.R.S., D.Sc., LL.D., Army Medical Service, and Captains A. E. Hamerton, D.S.O., and H. R. Bateman, Royal Army Medical Corps.

(Received September 18th,—Read November 26th, 1908.)

(From the Laboratory of the Royal Army Medical College, London.)

[Plates 1 and 2.]

About the middle of April, 1908, Dr. J. Rose Bradford, F.R.S., had handed over to him by Dr. Edington, F.R.S.E., a rabbit whose blood contained a trypanosome. Dr. Edington stated that he had inoculated the rabbit with blood from a horse he found at Zanzibar suffering from some obscure disease. This rabbit was handed over to one of us (D. B.) by Dr. Bradford for the purpose of keeping the strain alive and, if possible, identifying the species of trypanosoma.

The following notes have since been received from Dr. Edington. The trypanosome was found at Zanzibar, where no trypanosome has formerly been known. It occurred in a horse in a stable among others, of which none were infected. The animal was old, and had been many years in the place. At death the symptoms were like those in surra and nagana, but the spleen was not enlarged, nor was it coloured abnormally. The usual œdema was apparent and most marked in the sheath, up the abdomen, in the chest, and down the posterior limbs.

Dr. Edington inoculated a horse, an ox, and a goat successfully. The disease ran a sub-acute form in the original horse, but in the inoculated one it seemed rather more acute. Inoculated on February 18th, trypanosomes were seen in its blood on the 25th, and by March 1st the sheath was swollen. There was no real fever (102·2° F.) until February 28th, so that in this case the appearance of parasites preceded the fever. On March 7th it had greatly recovered, œdema had subsided, and the weakness of the preceding few days was recovered from. Dr. Edington left on March 8th, and fears the animal was destroyed, as they had no further vote for funds for food, &c.

A young ox, inoculated on February 15th, showed trypanosomes on the 27th. It had fever fairly high, but had recovered before he left, and trypanosomes were exceedingly few. A goat showed high fever, but its blood never showed trypanosomes at any time, although Dr. Edington hunted with very great thoroughness.

Two rabbits were inoculated, one subcutaneously and one intraperitoneally. The former was sent to Dr. Mesnil from Marseilles, but it has shown nothing. It was twice inoculated with big doses, one from a horse and one from an ox. The other Dr. Edington handed over to Dr. Bradford, and from this rabbit the trypanosome under consideration was obtained and studied.

On examining the rabbit's blood, the trypanosome was found to be a small one, with poorly-developed undulating membrane, and no free flagellum. The average length was only 13·5 microns.

Although it is impossible in some cases to name the trypanosomes from their shape and size alone, still it is evident that a trypanosome of this size, with no free flagellum, cannot be *Trypanosoma brucei, evansi, gambiense,* or several other species which need not be enumerated. The names of such small trypanosomes as *Trypanosoma nanum* (Laveran), *Trypanosoma congolense* (Broden), or *Trypanosoma dimorphon* (Dutton and Todd), at once occur to the mind.

No doubt the tendency in naming these hæmatozoa is to multiply unnecessarily the number of species. But, on the other hand, it is just as great a mistake to lump too many species together, as has been done. If there is some well-marked difference in two trypanosomes, even if alike in shape, such as their power of setting up disease in certain animals, their mode of spreading from the sick to the healthy—it may be in one by tsetse flies, in another by stomoxys, or tabanus, or by other means—then, naturally, it is of great practical use to distinguish them by different specific names.

Again, it might be argued, that if two trypanosomes were different morphologically, but had the same effect on animals, the same distribution and the same carrier, then the two varieties for practical purposes might be included in the same species.

For example, when we have to do with *Trypanosoma gambiense* we at once know that man is susceptible, that the carrier is *Glossina palpalis*, and that we must keep ourselves out of the area of distribution of this fly if we would escape infection. Theories in regard to the spread of sleeping sickness by mosquitoes, stomoxys, fleas, sexual intercourse, and such like, may, for practical purposes, be ignored. If it is *Trypanosoma brucei*, then we know man is not susceptible, but that we must keep our horses, cattle, and dogs out of the area of distribution of *Glossina morsitans*.

The three most important questions to be borne in mind, in classifying trypanosomes, are, what animals are they capable of infecting, the gravity of the infection, and, thirdly, what is the carrier? To these may be added the morphology of the trypanosome, its cultural characteristics, if any, and, if possible; cross-inoculation experiments. If these several facts could be set down for each trypanosome encountered in Africa, then some classification of the African species might be attempted. But it is only for a few species, such as *Trypanosoma gambiense* and *Trypanosoma brucei*, that we have all these data. Take, for example, the case of *Trypanosoma congolense* (Broden) and *Trypanosoma dimorphon* (Dutton and Todd)—most important trypanosome diseases. Laveran thinks they are distinct on account of a cross-inoculation experiment, but Broden himself, Rodhain, and Dutton and Todd all seem to lean to their really being one and the same species. With the data at our disposal at present it is impossible to come to a definite decision.

At the present time the classification of the pathogenic trypanosomes is in a state of chaos, and we have no desire to add to the confusion. Nevertheless, we think it will be well to give a description of Dr. Edington's trypanosome, as far as we have been able to study it, in view of the fact that we are starting at once for Uganda to continue the investigation of sleeping sickness.

MORPHOLOGY OF DR. EDINGTON'S TRYPANOSOME.

A. *Living, unstained.*

Dr. Edington's trypanosome in the fresh condition, as seen in a drop of blood from an infected guinea-pig or rat, appears short and stumpy in outline, about twice the diameter of the red blood corpuscles, among which it slowly moves, with, as a rule, its rapidly-vibrating flagellum in front. The posterior or non-flagellar extremity appears blunt and rounded off abruptly, while the anterior tapers off to a fine point. In the fresh preparation the undulating membrane is not much in evidence, though sometimes it can be seen thrown into waves. The contents of the cell are homogeneous, except for a small refractile body at the posterior extremity, which is evidently the micro-nucleus.

B. *Fixed and stained.*

Method of staining.—The method used for fixing and staining the trypanosomes is usually as follows. The blood-film while still moist is exposed to the vapour of a 4-per-cent. solution of osmic acid in distilled water, to which a drop of glacial acetic acid has been added, for 45 seconds. The cover-glass is then transferred to absolute alcohol for from five minutes to half an hour. It is then passed through grades of alcohol from 80 per cent. to 10 per cent. in distilled water. Twenty-five drops of Giemsa's stock stain (Grübler's) are now mixed with 25 c.c. of distilled water. The films are placed in this, face downwards, for 8 to 12 hours, then washed in distilled water, and rinsed quickly in solution of orange tannin (orange G. 1 per cent., tannin 5 per cent., in distilled water). When sufficiently decolorised, the films are washed in distilled water, dehydrated by passing through acetone, cleared in xylol, and mounted in canada balsam.

Dr. Edington's trypanosome when stained in this way appears of a pale puce colour with reddish-purple nucleus and micronucleus. The following detailed description must be understood to refer to this trypanosome as found in the blood of the white rat.

Length.—It is no easy matter to measure these small irregularly-shaped bodies, and doubtless the method of measurement used will govern to some extent the result. The method used by us is simply to draw a sharp outline of the trypanosome by means of a Zeiss camera lucida, at a magnification of 2,000 diameters, and then to measure along the middle line of the body by means of a pair of fine compasses, the points of which are separated 2 mm. Each step the compass takes is therefore equal to 1 micron. Twenty trypanosomes, taken as they come, are measured in this way in each specimen, and an average of the 20 measurements taken. The following table gives some of the results:—

Dr. Edington's Trypanosome.

No. of Experiment.	Day of Disease.	Method of Staining.	In Microns.		
			Average Length.	Maximum Length.	Minimum Length.
134, mouse	9	Giemsa ...	15·3	20·0	13·0
69, rat	30	Leishman ...	13·0	16·0	10·0
,, ...	30	Giemsa ...	13·9	17·0	10·0
,, ...	30	Methyl green	13·4	18·0	10·0
,, ...	30	Giemsa ...	15·0	18·0	13·0
,, ...	30	Leishman ...	13·5	17·0	11·0
84, rabbit	22	Giemsa ...	13·0	16·0	9·0
Guinea-pig	18	,, ...	12·5	16·0	8·0
166, dog	14	,, ...	13·0	16·0	10·0
		Average ...	13·6	17·1	10·4

For purposes of comparison measurements of *Trypanosoma dimorphon* and *Trypanosoma congolense* are given in the following tables:—

No. of Experiment.	Day of Disease.	Method of Staining.	In Microns.		
			Average Length.	Maximum Length.	Minimum Length.
		TRYPANOSOMA DIMORPHON.			
Mouse (Laveran and Mesnil).	?	Giemsa ...	13·8	17·0	12·0
116, rat (Breinl) ...	15	,, ...	13·8	16·0	11·0
,, ,, ...	9	Leishman ...	11·3	14·0	9·0
,, ,, ...	9	,, ...	12·6	15·0	11·0
Dog (Harvey, Sierra Leone).	?	,, ...	12·3	15·0	9·0
Cow (Smith, Sierra Leone).	?	,, ...	12·4	15·0	10·0
		Average ...	12·5	15·3	10·3
		TRYPANOSOMA CONGOLENSE.			
142, mouse	7	Giemsa ...	12·8	15·0	10·0
143, mouse	5	Leishman ...	11·5	14·0	10·0
152, rat	11	Giemsa ...	11·6	15·0	9·0
,, ...	11	,, ...	11·5	14·0	10·0
154, rat	19	,, ...	12·6	17·0	10·0
		Average ...	12·0	15·0	9·8

Breadth.—On an average the breadth at the widest part is 3 microns.

Shape.—Dr. Edington's trypanosome when stained is seen to be of a short and stumpy shape, somewhat reminding one of a miniature electric eel. The posterior extremity is, as a rule, blunt, or rounded or obtuse-angled, but sometimes, though rarely, it is prolonged into a sharp beak-like process. The anterior end tapers more or less, and ends in a short stout flagellum. The undulating membrane is narrow but distinct. The flagellum arises at or near the micro-nucleus and passes along the edge of the undulating membrane. There is no free flagellum, the protoplasm of the cell and the undulating membrane extending as far as the tip of the flagellum.

Contents of Cell.—The protoplasm, which is stained a pale puce colour, is homogeneous in structure.

Nucleus.—The nucleus is oval in shape, about 2·5 microns in length, and is situated at the centre of the trypanosome.

Micro-nucleus.—The micro-nucleus, centrosome, or kinetonucleus, is small, round, or rod-shaped, and is situated close to the posterior extremity. It stains more deeply than the nucleus.

Undulating Membrane.—The undulating membrane is narrow. As a rule it is straight and simple, and does not show much tendency to be thrown into folds.

Flagellum.—The flagellum stains intensely. It is well marked, and does not project beyond the protoplasm of the cell and the undulating membrane. Sometimes, in faintly-stained specimens, there is the appearance of a slight projection of the flagellum beyond the body; but, speaking broadly, this species of trypanosome may be said to have no free flagellum.

The conclusion to be drawn from a study of the morphology of Dr. Edington's trypanosome, *Trypanosoma dimorphon*, and *Trypanosoma congolense*, is, that the two first resemble each other very closely, whereas *Trypanosoma congolense* seems to be of a somewhat shorter and stouter form. It will also be seen that in the strain of *Trypanosoma dimorphon* used there is only one form, and that, the short or tadpole form described by Dutton and Todd. With regard to this, it may be of interest to quote some remarks of Dr. Breinl, to whom I am obliged for his courtesy in sending me this strain. He writes:—"With regard to *Trypanosoma dimorphon*, you are aware that some remarkable change has occurred in the strain between the time Drs. Dutton and Todd brought it back from Africa and we started work on it here. Whereas Drs. Dutton and Todd describe the long flagellated forms with the free flagella, Thomas and myself, Laveran and Mesnil, could not see these forms with a thin body and a long flagellum. The strain I send you in a rat is the original strain."

It is difficult to understand how this change in morphology has been brought about. It may be that Dutton and Todd were dealing with a double infection, of which one has died out. This point will require to be investigated on the spot.

Another matter for consideration is whether this name *Trypanosoma dimorphon* should be adhered to. It certainly seems a misnomer when applied to the strain figured above. If it should be decided to drop it, I think the compliment should be paid to Dr. Todd of naming it after him.

Inoculation Experiments on various Species of Animals.

The animals, in which the effect of the inoculation of Dr. Edington's trypanosome has been studied, have been horses, cattle, goats, monkeys, dogs, rabbits, guinea-pigs, white rats, and mice. The inoculations were made, as a rule, intraperitoneally. Inoculation experiments with *Trypanosoma dimorphon* are also given for purposes of comparison. These are printed in italics:—

No. of Experiment.	Source of Virus.	Period of Incubation, in Days.	Duration of Disease, in Days.	Remarks.
		HORSES.		
Edington	Unknown	Unknown	Unknown.	Dr. E. thinks ran sub-acute course.
"	Horse	7	"	Living after 18 days when Dr. E. left.
Dutton and Todd ...	*Natural infection.*	*Unknown*	*2·5 years, still alive.*	*Blood still infective.*
" ...	"	"	*1 year, still alive.*	*No record.*
Laveran	—	"	—	*Horse recovered.*

No. of Experiment.	Source of Virus.	Period of Incubation, in Days.	Duration of Disease, in Days.	Remarks.
CATTLE.				
Edington	Horse	12	Unknown	Animal looked well on 21st day.
Dutton and Todd...	—	*10·5*	*30*	*Two cattle.*
GOATS.				
Edington	Horse	Unknown	Unknown	Blood never showed trypanosomes up to 18th day.
Dutton and Todd...	—	*3·5*	*Well after a year.*	*Two goats.*
MONKEYS.				
174	Rat	3	22	Spleen enlarged. General glandular enlargement.
175	"	3	—	Still alive (Sept. 11, 1908).
Thomas and Breinl	—	*4 and 6*	*160 and 75*	*Two monkeys.*
Dutton and Todd...	—	*4*	—	*Two never became infected.*
DOGS.				
165	Rat	7	18	Spleen enormously enlarged.
166	"	9	15	" "
167	"	7	17	" "
179	"	7	14	Marked ulceration of stomach.
180	"	9	14	Spleen greatly enlarged.
181	"	7	14	" "
Thomas and Breinl	—	*4 to 8*	*10 to 19*	
Dutton and Todd...	—	*8*	*29*	*Average of 4 days.*
RABBITS.				
70	Rabbit	25	—	Still alive after 146 days.
84	"	10	—	Still alive after 136 days.
118	"	12	100	Spleen 4 inches long and much thickened.
119	"	15	—	Still alive after 103 days.
120	"	12	19	Spleen enlarged.
Thomas and Breinl	—	*9*	*Acute, 26—35; chronic, 78—157.*	
Dutton and Todd...	—	*13*	*53*	*One rabbit.*

No. of Experiment.	Source of Virus.	Period of Incubation, in Days.	Duration of Disease, in Days.	Remarks.
GUINEA-PIGS.				
82	Rabbit	25	134	Liver and spleen enlarged.
110	Rat	12	43	Liver and spleen greatly enlarged.
111	,,	19	43	,, ,,
124	,,	21	—	Still alive after 105 days.
125	,,	21	69	Liver and spleen enlarged.
126	,,	41	65	,, ,,
Dutton and Todd...	—	*6*	*30*	*Two guinea-pigs.*
Thomas and Breinl	—	*4 to 15*	*9 to 60*	
WHITE RATS.				
69	Rabbit	13	30	Typical *post-mortem* appearances.
83	,,	10	—	Killed for cultivation experiments.
83A	,,	11	—	,, ,,
127	,,	7	41	Usual *post-mortem* appearances.
128	,,	7	36	,, ,,
88	Rat ii	5	20	2nd passage through rat.
89	,, ii	5	—	Killed for cultivation experiments.
106	,, ii	5	—	,, ,,
107	,, ii	4	14	Typical *post-mortem* appearances.
108	,, ii	7	35	,, ,,
109	,, ii	5	28	,, ,,
102	,, iii	6	—	Killed for cultivation experiments.
102A	,, iii	7	37	Usual *post-mortem* appearances.
121	,, iii	4	37	,, ,,
122	,, iii	5	44	,, ,,
123	,, iii	4	—	Rat lost.
156	,, iii	7	20	Usual *post-mortem* appearances.
157	,, iii	7	23	,, ,,
Dutton and Todd...	—	*7*	*36*	
Thomas and Breinl	—	*4 to 7*	*7 to 42*	
MICE.				
132	Rat	10	24	
134	,,	4	11	
137	,,	10	11	
Dutton and Todd...	—	*5*	*16*	
Thomas and Breinl	—	*2 to 5*	*16 to 23* *37 to 130*	

Conclusion.—The results of these inoculation experiments with Dr. Edington's trypanosome and *Trypanosoma dimorphon* show that they act on the various animals employed in a strikingly similar manner.

CULTIVATION OF DR. EDINGTON'S TRYPANOSOME, *Trypanosoma dimorphon* AND *Trypanosoma congolense*.

In June, 1903, Novy and MacNeal first announced the successful cultivation of *Trypanosoma lewisi*. In the same year and in the following year they also succeeded in cultivating *Trypanosoma brucei* and *Trypanosoma evansi*. These gentlemen deserve the highest possible credit for this most difficult achievement, an achievement which most workers in this subject thought impossible. The amount of work they expended and the splendid intelligence and pertinacity with which they pursued their object, refusing to accept defeat, command the admiration of all their co-workers in this branch of biological science. Since then the trypanosomes of birds, frogs, and fish have been cultivated by the same and other workers; but these successes have only been made possible, as a rule, by the pioneer work of Novy and his assistants. Coming out of their work, mention may also be made of the very interesting and important observation made by Rogers when he grew Leishman's bodies in ordinary citrated blood into trypanosome-like flagellates.

One of the chief interests attaching to this cultivation of trypanosomes is that it may assist in separating the different species of these organisms. At the present time trypanology is in a state of chaos on account of this difficulty in differentiation. Many diseases of animals caused by trypanosomes have been reported from all parts of Africa, Arabia, India, the Philippines, Mauritius, &c., and it has often been found impossible to name the species of trypanosoma causing them with any approach to certainty.

As mentioned above, the usual method of separating the different species is by taking into consideration the morphology, the result of inoculation into animals, the cross-immunisation methods and serum diagnosis of Laveran and Mesnil, the mode by which the disease spreads from the sick to the healthy—by a tsetse fly, a stomoxys, a tabanus, or by contact, as in dourine—by the effect of various drugs, cultivation, &c.; and, as already stated, the effect the parasite has on animals and the mode of conveyance are probably, for practical purposes, the most important. But to assist in separating the various species, cultivation has been of use in the past, and, as the methods become perfected, will be of still greater use in the future.

The following description of the cultural characters of Dr. Edington's trypanosome exemplifies this, for, by comparing them with the cultural characters of other pathogenic species, a fairly shrewd guess at its classification may be made by this means alone. For the purpose of this comparison a compilation of the cultural characters of *Trypanosoma lewisi*, *Trypanosoma brucei*, and *Trypanosoma evansi* has been made from the writings of Novy, MacNeal, and Smedley.

It may be mentioned here that attempts have been made in this laboratory to cultivate these three species. The cultivation of the first was found to be a comparatively easy matter: but all attempts, and they were many, to cultivate the last two have, up to the present, failed, although Novy's instructions were carefully followed.

Cultivation Medium used.

The blood-agar medium used was made according to instructions kindly sent by Professor Novy. These need not be repeated here, as the details are fully given by Novy and MacNeal in various papers.

CULTURAL CHARACTERS OF *Trypanosoma lewisi.*

A. *Living, unstained.*

Size.—Varies considerably in size. Some are not more than 1 or 2 microns long, not including the flagellum. Others are about the diameter of a red blood corpuscle, while the usual length of the spindle-shaped cells is 15 to 20 microns. Some trypanosomes can be found at times which are 50 to 60 microns long. The greatest variation in size is found in young cultures.

Shape.—*Trypanosoma lewisi* varies greatly in shape, as well as in size. Round, pear-shaped, fusiform and slender forms are present in the cultures. The round forms are usually found in old cultures, and are probably involution forms.

Contents of Cell.—The protoplasm in *Trypanosoma lewisi*, especially in young cultures, is bright, glistening, and apparently homogeneous in structure in the fusiform and slender forms.

Undulating Membrane.—Not present as far as can be seen. The movement of these cultural forms appears to be entirely due to the rapid motion of the flagellum.

Flagellum.—These forms possess, as a rule, a long free flagellum. In the slender forms this is sometimes twice the length of the body.

Motion.—The single, slender, cultural forms of *Trypanosoma lewisi* are very active, and dart across the field of the microscope in a straight line. In older cultures the round and other involution forms do not, as a rule, show more than a slight swaying movement.

Colonies or Aggregations.—Growth commences in a first generation about the fifth day by the appearance of small rosettes composed of a few trypanosomes. The Colonies rapidly grow, so that on the following day masses of wriggling trypanosomes may be seen. These aggregations of twenty or more are attached by their flagella. They grow larger and larger until, about the twenty-fourth day, they are apparent to the naked eye, and consist of many thousands of trypanosomes.

B. *Fixed, stained.*

Protoplasm.—Homogeneous, as a rule. Vacuolation is rare, but sometimes a large highly-refractile vacuole is seen.

Nucleus.—Round or oval in shape. Situated centrally or at the junction of the anterior and middle thirds.

Micro-nucleus.—Is placed either close to the nucleus or at a variable distance anterior to it. In the free forms it is never seen lying posterior to the nucleus. As a rule, it is a rod-shaped structure, lying transversely to the long axis of the trypanosome.

Flagellum.—Arises from the vicinity of the micro-nucleus. The free flagellum is often two, three or four times the length of the body of the trypanosome.

Undulating Membrane.—In the cultural form of *Trypanosoma lewisi* this structure is apparently absent.

Colonies or Aggregations.—There is little to add to the description of the trypanosomes and of their arrangement in Colonies. Stained preparations show that the trypanosomes sometimes possess very long flagella. Novy and MacNeal* have not apparently succeeded in staining the flagellum in their preparations, though they noted the position of the centrosome. They expressed the opinion that the end of the trypanosome pointing towards the periphery of the Colony was the anterior extremity, and that from it a flagellum would arise if the cultural conditions were perfected (Smedley).

Measurements of the Cultural Forms of Trypanosoma lewisi.

Pear-shaped Forms.—(1) Body, 3·6 to 4·4 microns long, and nearly as broad. (2) Flagellum, two to four times the length of the body.

Spindle-shaped Forms.—14 to 16 × 2·4 to 3·5 microns, flagellum not included.

Smaller and larger forms are frequently found.

The adult parasitic form of *Trypanosoma lewisi* measures 24 to 25 × 1·5 microns (Laveran and Mesnil) (Smedley).

CULTURAL CHARACTERS OF *Trypanosoma brucei.*

A. *Living, unstained.*

Size.—Shows less variation in size than *Trypanosoma lewisi*, and averages 15 microns in the living condition. Smaller than those found in the blood.

Shape.—Do not vary much in shape, and closely resemble the forms found in the blood (Smedley).

Contents of Cell.—Show one or two very large, bright, and highly-refracting globules, usually placed near the anterior or flagellar end, in the otherwise homogeneous colourless cell. In size the globules may attain 1 micron. At times the number of these globules is increased, as when the culture is kept at 34° C. The presence of numerous large, highly-refractile globules in the cultural forms of *Trypanosoma brucei* is attributed by Novy and MacNeal to degeneration of the organisms, owing to imperfection of the culture medium. These globules become more numerous as the age of the culture advances. Do not seem to alter in position or shape if kept under observation for several hours. Resist staining completely. Laveran and Mesnil suggest that the globules are of the same nature as the refringent, unstainable granules found in *Trypanosoma rotatorium*.

Undulating Membrane.—No detailed description available.

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* 'Cultivation of *Trypanosoma brucei*,' p. 28.

Flagellum.—The flagellum in the living cell is by no means as distinct and as long as that of *Trypanosoma lewisi.*

Motion.—The motion of *Trypanosoma brucei* is slow and wriggling, and only exceptionally is a slowly-progressive form observed. The wave-motion slowly passes along the thick, undulating membrane, and gives the appearance of a spiral rotation to the entire cell. Scarcely departs from its place (Novy). In a young culture the trypanosomes are found to possess very active movements. Sometimes they advance across the field moderately quickly, but their rate of movement is always much slower than that of the rat trypanosomes, whose flagella are longer and more rapid in motion (Smedley).

Colonies or Aggregates.—Occurs in groups or rosettes. Rarely forms masses of more than 10 to 20 cells. The individuals are long, narrow, and show the peculiar writhing motion. The flagella are directed outwards, and the appearance of the whole may be compared to the snakes on a Medusa head. The stellate group with the bright, refracting globules within the cells, suggests a jeweller's " sun burst " (Novy). The active movements of the trypanosomes, and the large glistening vacuoles with which they are studded, give these colonies a singularly beautiful appearance (Smedley).

B. *Fixed, stained.*

Protoplasm.—The protoplasm invariably contains a few deeply-stained granules of a red or violet colour. The vacuoles are seen as clear circular spaces with sharply-defined outlines in stained preparations (Smedley).

Nucleus.—Round or oval in shape; and in older forms it breaks into masses of cromatin, which are found distributed through the protoplasm of the cell (Smedley).

Micro-nucleus.—This is much smaller than in *Trypanosoma lewisi;* it is usually circular, but sometimes elongated. It stains a deep red or purple colour, and it is sometimes difficult to distinguish it from the other granules. It is generally found close to the vacuole; sometimes it lies close to the nucleus, but it is nearly always posterior to the latter structure (Smedley).

Flagellum.—Takes a tortuous course along the free border of the undulating membrane, and projects for a short distance from the anterior extremity (Smedley).

Undulating Membrane.—No detailed description given.

Colonies or Aggregates.—Most of the flagella are directed in an outward direction. It is rare to find Colonies of a large size (Smedley).

Measurements of the Cultural Forms of Trypanosoma brucei.

Length, including flagellum, 18 to 23 × 2·5 to 3·5 microns. Length of free flagellum, 3 to 5 microns. Diameter of vacuoles, 1 to 2 microns. The adult parasitic forms of *Trypanosoma brucei* measure, in the blood of rats, 26 to 27 × 1·5 to 2·5 microns (Laveran and Mesnil) (Smedley).

CULTURAL CHARACTERS OF *Trypanosoma evansi*.

A. *Living, unstained.*

Size.—The body of one large individual measured 21 microns, while the flagellum was 28 microns in length.

Shape.—The slender fusiform body terminates at one end in a delicate flagellum. The posterior end, especially when blunt, showed a rod-like tip or stylet, which varied from 2 to 4 or even 6 microns in length. As the cultures aged, pear-shaped or spherical, highly granular, involution forms appeared. In the former type, measuring about 3 by 5 microns, the end was often provided with a flagellum, 10 to 15 microns long, which still showed a slow lashing movement, though the cell itself was motionless. The spherical forms varied from 4 to 9 microns in diameter, were granular, and often showed a remnant of the flagellum as a short, stiff, motionless whip. These involution forms, as in the case of *Trypanosoma lewisi* and *Trypanosoma brucei,* eventually gathered into large groups or masses, which at times filled the field of an immersion lens. Later on, the round bodies broke up into masses of very minute granules.

Contents of Cell.—Presence and peculiar arrangement of granules within the cells, and a distinct yellowish or greenish colour of the granules and of the contents. Large numbers of small granules or globules, which vary from 0·3 to 0·5 micron in diameter. These globules, as well as the contents of the cell, possess a decided yellowish or greenish colour, and appearance quite unlike that of either *Trypanosoma lewisi* or *Trypanosoma brucei.* The globules are usually massed in the anterior-third of the cells—that is, at the base of the flagellum, and only a few isolated granules are scattered through the remainder of the organism (Novy and MacNeal).

Undulating Membrane.—Is not recognisable in the living organism.

Flagellum.—Usually as long and often even longer than the cell itself.

Motion.—All single and actively motile, traversing the field of the microscope at great speed. Travel with the flagellum in rear or in front.

Colonies or Aggregates.—Entire absence of the groups or rosettes, which are so characteristic of the cultures of *Trypanosoma lewisi* and *Trypanosoma brucei.* The trypanosomes were all single and actively motile.

Measurement of the Cultural Forms.

Length, including flagellum, 25 to 50 by 1·5 to 2·5 microns.

CULTURAL CHARACTERS OF DR. EDINGTON'S TRYPANOSOME.

A. *Living, unstained.*

No difficulty is found in cultivating Dr. Edington's trypanosome. As early as the second day, if kept at 25° C., it is found to have greatly increased in numbers. The single individuals are in active motion, the flagellum wildly waving, while the body slowly moves among the corpuscles. Many dividing forms are seen with two or three flagella. Masses or aggregations are also seen varying in size, from those composed of a dozen individuals to those occupying a fifth of the field. The aggregation-forms are all writhing and squirming, while the flagella at the periphery are frantically waving. This incessantly moving mass, dotted over as it is with many small bright vacuoles, makes a curious and beautiful microscopic object when brightly illuminated.

On the third day the trypanosomes have multiplied to an extraordinary extent. Huge aggregations are now seen, each filling up several fields of the microscope. The individual trypanosomes are still actively motile. Single, double, and small aggregations are also seen.

By the seventh day they have reached the height of their growth and begin to degenerate.

After the twelfth day living forms can no longer be recognised in the culture tubes.

Size.—Dr. Edington's trypanosome, examined in the fresh living condition, varies considerably in size. Some of the large forms measure 32 microns in length, whereas the smaller are only half that length, or even shorter.

Shape.—So also in regard to shape, these cultural forms vary extremely. Round, oval, pear-shaped, and irregular forms are seen. Slender forms shaped like ordinary trypanosomes, with a beak or rostellum at one end, a fairly thick flagellum at the other, and furnished with an undulating membrane, are fairly common. Large irregular masses of any shape, furnished with one or more flagella, are also frequent.

Contents of Cell.—These cells have a remarkable appearance, as they are filled with highly refractile granules, large in size, round in shape, and numerous.

Undulating Membrane.—The round, oval, and pear-shaped forms do not appear to possess an undulating membrane, whereas the long, slender forms, as also the huge fish-shaped or octopus-like forms, often show well-marked undulating membranes.

Flagellum.—The flagella in these living unstained cultural forms are thick and coarse, and differ markedly from the slender structures usually associated in the mind with trypanosomes. Just as in the parasitic forms found in the blood, it is evident that the protoplasm of the body extends to the tip of the flagellum giving rise to this thick stumpy appearance.

Motion.—The slender forms are active and swim fairly quickly across the field. The large, irregular forms are stationary, but exhibit actively wriggling flagella and amœboid movements of the body substance.

Colonies or Aggregates.—Colonies or aggregations of 10 to 20 individual cells are common. The cells are arranged irregularly. Some of their flagella may be directed outwards, while others are seen entangled in the mass and feebly wriggling. On the third day these aggregations may be seen as large as three to five fields of the microscope, and must be composed of many thousands of individual trypanosomes.

B. *Fixed, stained.*

Method of Fixing and Staining.—The cultural forms of Dr. Edington's trypanosome were either prepared by mixing a drop of the cultivation fluid with fresh serum, spreading on a slide, and staining by Leishman's modification of Romanowsky's method, or the fluid was spread on a slide, fixed by osmic acid and stained by Giemsa, and then treated with orange tannin to differentiate the various structures.

In Leishman-stained preparations the protoplasm of the cells is stained a pale blue, the nuclei and irregular masses of chromatin reddish or pink, while the vacuoles stand out as unstained spaces with sharply-defined margins. In Giemsa-stained preparations, on the other hand, the protoplasm is stained a pale puce colour, while the chromatin material is stained reddish purple.

Protoplasm.—The protoplasm of the cell is homogeneous, but contains irregular-shaped granules and masses of chromatin-staining material. There are also present numerous well-marked vacuoles of various sizes, which are unstained, and, as mentioned above, highly refractile.

Nucleus.—The nuclei are of every form and shape, and often broken up into irregular masses.

Micro-nucleus.—The micro-nuclei are irregularly placed; in some cells are not easily distinguishable from other granules contained in the protoplasm, but in many are clearly seen as deeply-staining bodies, round or rod-shaped, in close connection with the point of origin of the flagella.

Flagellum.—The flagella are, as a rule, thick and fleshy. In the irregular forms they appear to spring from any part of the shapeless mass of protoplasm, and in any direction.

Undulating Membrane.—The undulating membrane is also characterised by its extreme irregularity. In many cells it appears to be absent, while in others it is well marked, broad, and thrown into folds.

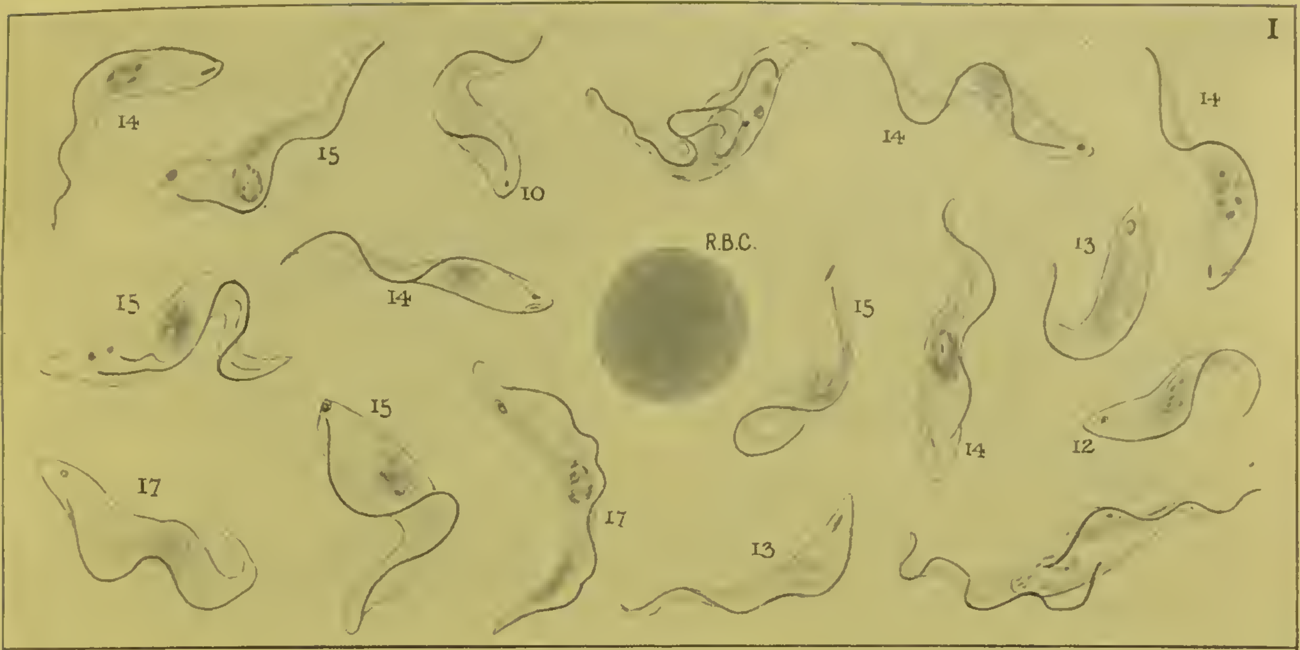
Colonies or Aggregations.—The individual trypanosomes which go to compose the large aggregations are as a rule short and stumpy in form, with oval-shaped nucleus and short stumpy flagellum. They are of irregular shape and size, and are placed without any seeming order.

CULTURAL CHARACTERS OF *Trypanosoma dimorphon* (DUTTON AND TODD).

It is unnecessary to describe in detail the cultural characters of this trypanosome, as they agree exactly with those of Dr. Edington's.

CULTURAL CHARACTERS OF *Trypanosoma congolense* (BRODEN).

Several attempts were made to cultivate *Trypanosoma congolense,* but none of them were very successful. There is certainly not the rapid growth of this trypanosome which distinguishes Dr. Edington's trypanosome and *Trypanosoma dimorphon.* It is only after a long search that individual trypanosomes can be found in the preparations. There is no formation of masses or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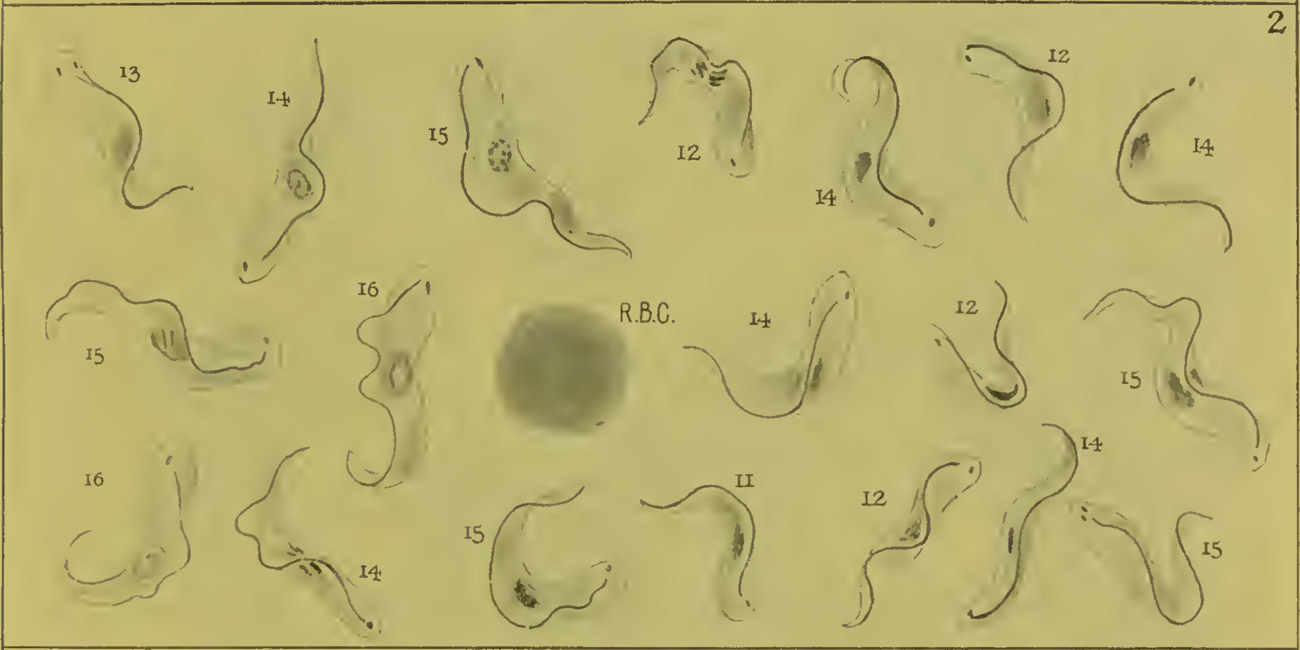
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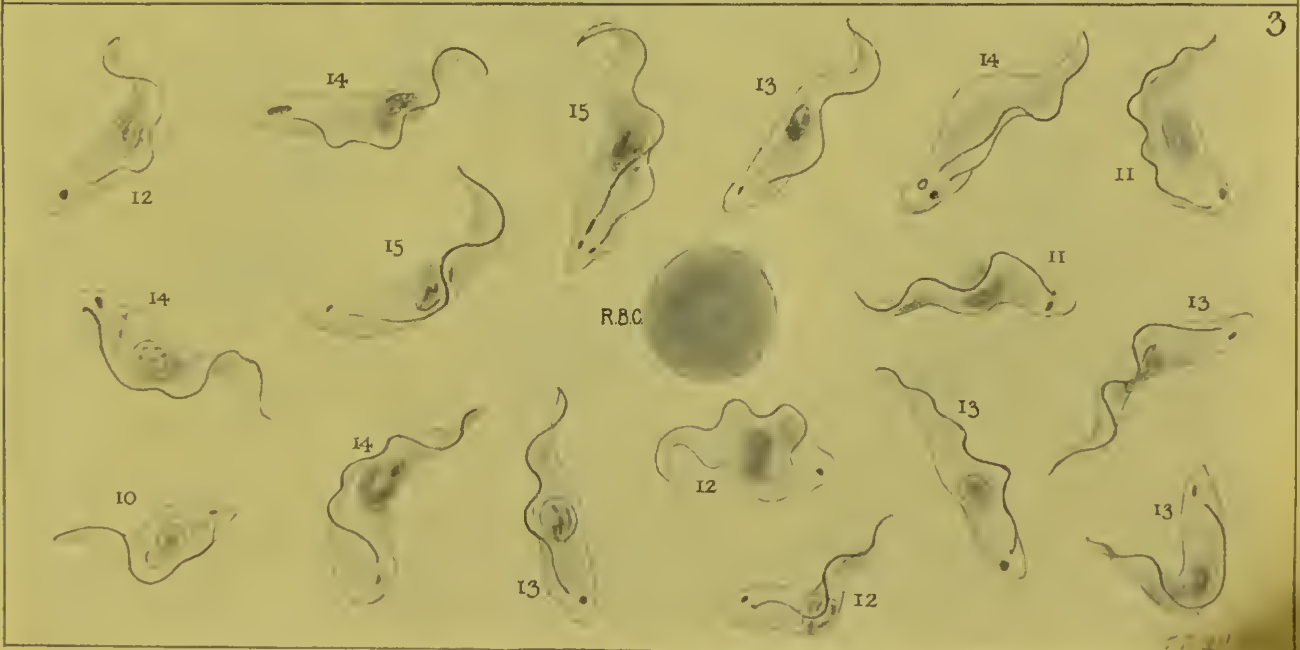
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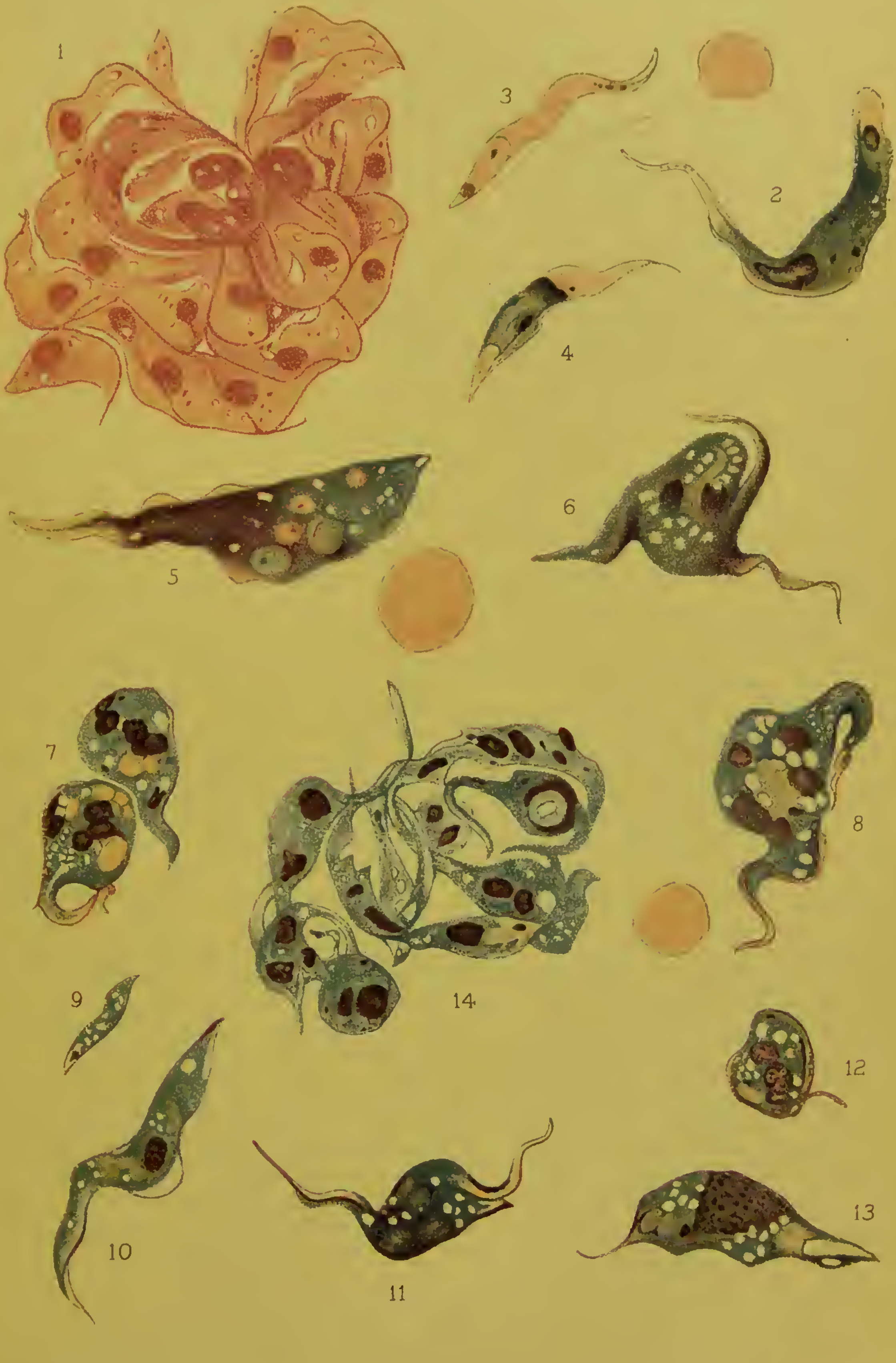


[illegible] & SON [illegible] WALLTON [illegible]

aggregations filling several fields of the microscope as in the others. It is difficult to say whether there is any real multiplication or not. All that can be said is that, for about eight days, living trypanosomes can be seen. At first these are shaped like the ordinary trypanosomes found in the blood, only larger and swollen in appearance; but by the fifth and following days these change into most irregular and fantastic shapes. Nothing living could be seen after the eighth day. This cultivation experiment would therefore seem to strengthen Dr. Laveran's opinion that *Trypanosoma dimorphon* and *Trypanosoma congolense* are distinct species.

Conclusion.

The conclusion arrived at is that Dr. Edington's trypanosome from Zanzibar is probably Dutton and Todd's *Trypanosoma dimorphon*. One link in the chain of evidence, however, is wanting, and that an important one—the identity or non-identity of the carrier.

DESCRIPTION OF PLATES.

PLATE 1.

This plate represents the shape and size of the three Trypanosomes, viz. :—

1. Dr. Edington's trypanosome. From blood of rat. 30th day of disease. Stained Giemsa. × 2000. *See* p. 26.
2. *Trypanosoma dimorphon.* From blood of rat. 15th day of disease. Stained Giemsa. × 2000. *See* p. 26.
3. *Trypanosoma congolense.* From blood of mouse. 7th day of disease. Stained Giemsa. × 2000. *See* p. 26.

PLATE 2.

FIG. 1.—Part of an aggregation of Dr. Edington's trypanosomes after 5 days' growth. Stained Giemsa. × 2000.

FIGS. 2–4.—Dr. Edington's trypanosome after 6 days' growth. Stained Giemsa. × 2000.

FIGS. 5–14.—Cultural forms of Dr. Edington's trypanosome after 7 days' growth. Stained Leishman. × 2000.

[*Reprinted from the* PROCEEDINGS OF THE ROYAL SOCIETY,
B. Vol. 81.]

31. TRYPANOSOMA INGENS, n. sp.

By Colonel Sir DAVID BRUCE, C.B., F.R.S., Army Medical Service; Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, Royal Army Medical Corps; and Captain F. P. MACKIE, Indian Medical Service.

(Received April 30,—Read May 20, 1909.)

(Sleeping Sickness Commission of the Royal Society, 1908—09.)

[PLATE 3.]

This is such an extraordinary looking parasite that the Commission thinks it deserves a short preliminary note, a name, and to be figured.

The name is taken from Virgil's description of the Cyclops, *informe, ingens*. It was first discovered in the blood of a reed-buck on February 13, 1909, at Namukekera, Uganda (lat. 0° 40′ N.; long. 32° 15′ E.), the estate of the Uganda Company, Limited; then in a bush-buck, and lastly in an ox. The wild animals and the cattle feed in the same pastures, so that it is not remarkable that the oxen should become infected.

At present it is not known what the carrier is, and this will probably be a difficult thing to determine. Collections of the blood-sucking flies and ticks are being made on the Namukekera Estate, and this may lead in time to the discovery of the carrier. Up to the present the following list includes all the blood-suckers found in this particular district:—

Chrysops distinctipennis, Austen.
Stomoxys calcitrans, Linn.
Stomoxys nigra, Macq.
Tabanus tæniola, Pal de Beauv.
Hæmatopota unicolor, Ricardo.
Hæmatopota, sp. nov.
Hæmatopota brunnescens, Ricardo.

Trypanosoma ingens, when seen alive in a fresh preparation, moves slowly and deliberately across the field of the microscope, with a fine rippling, or at times a broader undulating movement.

In stained preparations this huge trypanosome may measure as much as 122 microns, and even then it is lying in such a formless huddled-up way among the red blood corpuscles that it looks capable of stretching out to a much greater length. The other specimens figured measure 72, 77, 88, and 82 microns. The breadth is 7 to 10 microns.

The micronucleus is small and round. It measures about a micron in diameter. It lies posterior to, and quite close to, the nucleus. From it, in well-stained specimens, a well-marked, though narrow, undulating membrane arises, which runs to the anterior extremity and ends in a free flagellum.

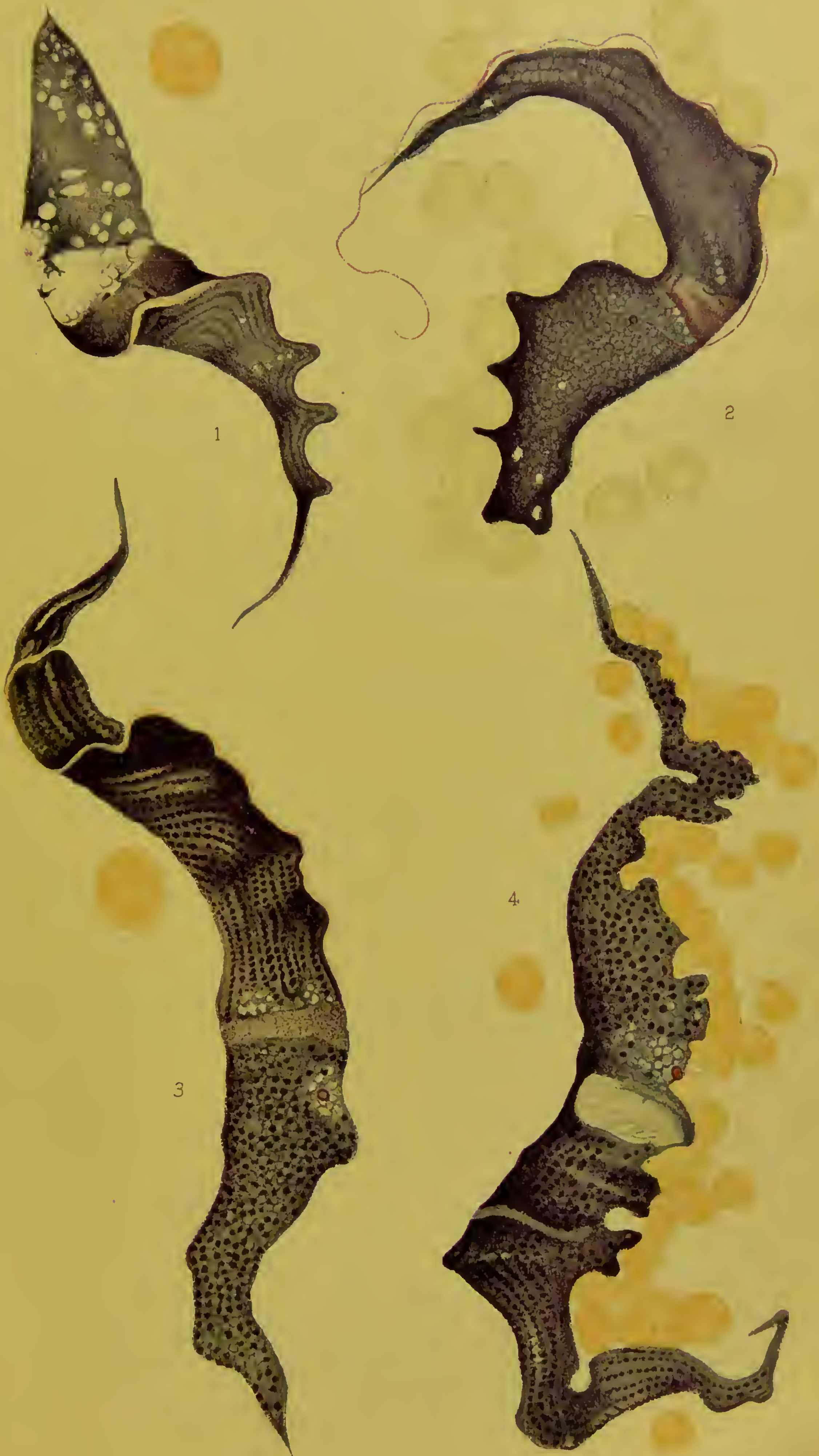
The nucleus is oval in form, and lies across the body. It is situated nearer the posterior end than the anterior, and in our specimens has stained a pale pink.

The body substance is markedly granular behind the nucleus, while in front the structure described as myonemes is particularly well marked.

More minute measurements of one of these trypanosomes are as follows:—

Posterior end to micronucleus	18
From micronucleus to nucleus	4
Nucleus: long diameter, 8 microns; short diameter	4
Nucleus to anterior end	40
Free flagellum	17
Total	83

It is unnecessary in this preliminary note to go more fully into the structure of this trypanosome, or to describe it at greater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length. An examination of the coloured drawings reproduced in Plate 3 will give a more distinct idea of its appearance than any written description.

The drawings were made by Lady Bruce, R.R.C. Figs. 1, 3, and 4 are from reed-buck, fig. 2 from the ox. All are magnified 2,000 and stained Giema.

[*Reprinted from the* PROCEEDINGS OF THE ROYAL SOCIETY, B. Vol. 81.]

32. FURTHER RESULTS OF THE EXPERIMENTAL TREATMENT OF TRYPANOSOMIASIS: BEING A PROGRESS REPORT TO A COMMITTEE OF THE ROYAL SOCIETY.

By H. G. PLIMMER, F.L.S., and W. B. FRY, Captain R.A.M.C.

(Communicated by J. Rose Bradford, M.D., Sec. R.S. Received June 28, 1909.)

The following results are a continuation of the work of which summaries have already appeared in the "Proceedings of the Royal Society."*

These experiments have been carried out, with the same strain of Surra as was used before, at the Brown Institution and the Lister Institute.

A.—*Condition of the Animals living at the Date of the Completion of the Tables in the last Paper.*

Rats treated with Sodium Antimonyl Tartrate, 1 *per cent.* (*p.* 478).

No. 7 died 428 days after inoculation.
,, 32 ,, 409 ,,
,, 35 ,, 371 ,,

Rats treated with Sodium Antimonyl Tartrate, 5 *per cent., in Colonel Lambkin's Medium* (*p.* 482).

No. 13 died 216 days after inoculation.

Rats treated with Antimony (metal), 5 *per cent., in Colonel Lambkin's Medium* (*p.* 483).

No. 10 died 205 days after inoculation.
,, 17 ,, 367 ,,
,, 25 ,, 385 ,,
,, 27 ,, 399 ,,
,, 29 ,, 360 ,,

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* B, vol. 79, 1907, pp. 500–516 ; and B, vol. 80, 1908, pp. 1–12 and 477–487.

Rats treated with Lithium Antimonyl Tartrate, 0·25 per cent (*p.* 485).

No. 4 died 145 days after inoculation.
,, 5 ,, 229 ,,
,, 6 ,, 257 ,,
,, 8 ,, 241 ,,
,, 10 ,, 209 ,,

Most of the above rats died from cold; none of them died from the disease, and no trypanosomes were found in their blood or organs, and inoculations made therefrom were entirely negative.

B.—*Further Experiments.*

Rats treated with Lithium Antimonyl Tartrate, 1 per cent.

A further series of experiments has been carried out with this substance on a large number of rats, giving four doses of 4 to 5 minims (according to weight) of a 1 per cent. solution subcutaneously, a dose being given every other day. Practically by this method every rat can be cured. They have lived for varying periods, up to 249 days, and in no case have trypanosomes been found after death in the blood or in the organs. No rat has died of the disease, and in no case thus treated has there been a recurrence. The results have therefore been more constant than those attained with sodium or potassium antimonyl tartrates. The treatment was begun on the third or fourth day after inoculation; it will be seen below that when it is left until the number of trypanosomes in the peripheral blood is very great, although they may be driven out of the blood, it does not cure: so that the time at which treatment is commenced is of considerable importance.

It has also been given intravenously in rabbits, but with far less effect than when given subcutaneously. The elimination in this case is very rapid, to which fact we attribute its comparatively feeble action.

Rats treated with Lithium Antimonyl Tartrate on the Fifth or Sixth Day of the Disease.

The blood at this period of the disease is swarming with trypanosomes, and experiments were made in order to see what effect this salt of antimony would have upon the disease at this period. If one dose of 5 minims of a 1 per cent. solution be given the rats die on the seventh day, so that little or no effect is produced If two such doses be given, one on the fifth and one on the sixth day, the average time of death in 10 rats was 19½ days, and living trypanosomes were found in the blood at death. When four doses were given, one on each day from the fifth to the eighth, the time in three rats was lengthened to 81 to 86 days: in one of these even living trypanosomes were found in the blood after death. By comparing these results with those mentioned in the former section it will be seen that the time at which the administration of the drug is begun is of importance, as well as the number of doses. The animals stand the best chance of cure when no recurrences take place, and this is best ensured by the method described in the previous section.

Further experiments made with Rats treated with Antimony in order to find out in what Organs the Trypanosomes are latent.

Following on the experiments made on rats treated with sodium antimonyl tartrate, with the view of finding out where the trypanosomes are latent, and recorded in the last paper,* a further series of experiments has been made on rats inoculated with Surra, which is more amenable to treatment with antimony than the Nagana used in the former series, and completely treated (that is, given a curative series of doses) with lithium antimonyl tartrate: this, as stated in the paper referred to, appears to be the most active of this variety of salt.

Seven rats were treated with four doses of 5 minims of a 1 per cent. solution of lithium antimonyl tartrate, and they were killed in succession, one on the 6th, 7th, 10th, 14th, 16th, 22nd, and 30th days after the last dose. The livers and bone-marrows were made into an emulsion with the minimum quantity of 0·89 per cent. salt solution, and 1 c.c. of the emulsions of these organs and 1 c.c. of heart's blood was injected separately into other rats. The results were entirely negative. Microscopic preparations were made of the material injected and no organisms were seen, and none of the sub-inoculations gave a positive result.

Experiments made in order to see if any Protection was afforded by Initial Treatment with Antimony.

A series of six rats was treated with four doses of 5 minims of a 1 per cent. solution of lithium antimonyl tartrate, one dose every other day in the same manner as when given for curative purposes. They were then inoculated with Surra, one on the first day after the completion of the treatment, and one on the 2nd, 4th, 5th, 9th, and 10th days after. They all died on the 5th or 6th days after inoculation, just as untreated rats would have done, so that antimony in this very soluble form is no protective use in rats, owing most probably to its rapid elimination.

The blood of an uninfected rat treated as above has also been used in the *in vitro* experiments recorded below.

Rats treated with Sodium Antimony Lactate and with Antimony Sodium Calcium Lactate.

Through the kindness of Messrs. von Heyden we have been enabled to make some experiments with the above compounds. The sodium antimony lactate contains 26 per cent. of antimony.

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* 'Roy. Soc. Proc.,' B, vol. 80, p. 487.

and the antimony sodium calcium lactate 17 per cent., so they are both much weaker in antimony than the tartrates which we have used. By the addition of a small quantity of lactic acid we were able to get a 1 per cent. solution of both salts, and in this strength the solutions were not very irritating, but neither with rats nor with larger animals are they as effective as the tartrates or the metal.

The following table shows the results obtained with sodium antimony lactate 1 per cent.

Average duration of untreated disease 6·9 days:—

Rats of 150 to 200 Grammes Weight.	Number of Doses, and Quantity.	Recurrences.	Lived.	Remarks.
1	2 of 4 minims	0	9 days	Died from enteritis.
2	4 of 4 ,,	0	20 ,,	Died from retained fœtus.
3	4 of 4 ,,	1	37 ,,	
4	5 of 4 ,,	1	46 ,,	
5	6 of 4 ,,	2	100 ,,	(No trypanosomes found in any of these rats after death.)
6	4 of 5 ,,	0	74 ,,	
7	5 of 5 ,,	1	48 ,,	

The following table shows the results obtained with antimony sodium calcium lactate 1 per cent.

Average duration of untreated disease 6·9 days:—

Rats of 150 to 200 Grammes Weight.	Number of Doses, and Quantity.	Recurrences.	ived.	Remarks.
1	3 of 4 minims	1	83 days	No trypanosomes found *post-mortem.*
2	4 of 4 ,,	0	25 ,,	Living trypanosomes found *post-mortem.*
3	5 of 4 ,,	2	68 ,,	No trypanosomes found *post-mortem.*
4	6 of 4 ,,	2	45 ,,	,, ,, ,,
5	5 of 5 ,,	2	64 ,,	,, ,, ,,
6	6 of 5 ,,	2	68 ,,	,, ,, ,,
7	8 of 5 ,,	2	57 ,,	,, ,, ,,
8	4 of 7 ,,	0	131 ,,	,, ,, ,,

On dogs the effect was very much less marked than on rats, and an effective dose became inconveniently large.

The following experiments show the relatively greater time taken for these salts to act, as compared with the sodium or lithium antimonyl tartrates, which drive all the trypanosomes from the peripheral blood in about an hour after the dose.

A Surra rat was taken on the fourth day, when the trypanosomes are numerous in the blood, and 5 minims of a 1 per cent. solution of sodium antimony lactate were injected.

Blood was taken	and showed the following:
½ hour after injection...	Trypanosomes affected by the drug: are extremely active, and show a tendency to swell.
1 ,, ,, ...	Very few normal trypanosomes to be seen: nearly all are swollen and spherical in shape (=" battledores "). Still large numbers.
1½ hours ,, ...	Much smaller number of trypanosomes to be seen: a few " battledores ": a few motionless ones, and one or two normal forms.
2 ,, ,, ...	" Battledores " have all disappeared: one or two slowly moving normal forms seen.
2½ ,, ,, ...	Ditto.
3½ ,, ,, ...	No trypanosomes found.

A similar experiment made with a rat treated with antimony sodium calcium lactate yielded practically the same result. Further experiments made with these drugs *in vitro* will be mentioned later.

Experiments made with Antimony (Metal in state of finest Division) suspended in various Oily Media.

Since the curative results following treatment with the metal antimony* suspended in Colonel Lambkin's medium seemed promising, many trials have been made with the metal suspended in other oily media, such as olive oil, cod liver oil, lanolin, egg-yolk, &c., in order, if possible, to obviate, or at any rate reduce, the extremely irritating properties of the metal, which seriously interfere with its practical use.

In olive oil a 5 per cent. suspension was used: with one dose of 3 minims Surra rats lived for 15 days, and died with living trypanosomes in their blood. Seventeen Surra rats were given one dose of 5 minims on the fourth day of the disease, and they lived from 41 to 133 days: in these there were no recurrences, nor were trypanosomes found after death, and sub-inoculations were in every case negative. Six Surra rats were treated with the same dose in order to observe the time taken for the complete disappearance of the trypanosomes from the blood.

Blood was taken	and showed the following:
½ hour after injection...	Trypanosomes very active.
1 ,, ,, ...	As numerous: show evidences of swelling.
1½ hours ,, ...	Still numerous: nearly all swollen: some " battledores."
2 ,, ,, ...	Very few forms found: all " battledores."
2½ ,, ,, ...	No trypanosomes seen.

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* 'Roy. Soc. Proc.,' B, vol. 80, p. 483.

Two Surra rats were taken on the fifth day, when the blood was swarming with trypanosomes, and 6 minims were given. Two and a-half hours after the rats were killed, and smears were made from the lungs, liver, spleen, kidney, bone-marrow, heart's blood, and brain. In none of the specimens could a trypanosome be found after prolonged examination.

This oil was also given to several rats upon recurrences after treatment with small doses of the lactates mentioned above: in these cases the effect was much less marked, even although the number of trypanosomes in the blood was much less than in the rats treated for the first time. This accords with our general experience that recurrences are much more difficult to deal with than the initial infection, and this applies to all the drugs we have tried.

A suspension in cod liver oil took four hours to drive the trypanosomes out of the peripheral blood.

The suspension in egg-yolk appeared to act in rats better than any other; in dogs, however, the results were variable; sometimes strikingly good, at others no better than the other mixtures: sometimes causing great irritation and sloughing, sometimes not causing any irritation at all. We have rats alive for more than 120 days after inoculation, with no recurrences, after one dose.

An experiment was made to see how long one dose took to drive the trypanosomes out of the blood. A Surra rat on the fourth day was treated with 5 minims of a 5 per cent. suspension.

Blood was taken	and showed the following:
¾ hour after injection...	Trypanosomes much affected, but not decreased. Many "battledore" forms.
1¼ hours ,, ...	Trypanosomes reduced in numbers: all swollen and "battledore" forms: very little movement.
2½ ,, ,, ...	No trypanosomes found.

Experiments with Quassia.

Dr. Guillemard, of Cambridge, suggested that quassia, on account of its known poisonous effects on some of the lower forms of life, should be tested for its trypanocidal qualities. A series of experiments was therefore undertaken on rats.

Six Surra rats were treated on the third and following days of the disease with a 5 per cent. solution of the pharmacopœal extract of quassia: they were given three doses subcutaneously—5 minims on the third day, 10 minims on the fourth, and 10 minims on the fifth day. The trypanosomes were entirely unaffected, and the animals died on the sixth—seventh day. Another series of 12 Surra rats was treated with a two hours' decoction of quassia-wood made with the minimum amount of water. Of this three doses were given—5 minims on the third day and 10 minims on the fourth and fifth days. The trypanosomes in these rats were also entirely unaffected, and the animals died on the sixth—seventh day. It was also tried intravenously in rabbits in doses of 30 minims of the decoction: no effect was produced, and the rabbits died on or about the 42nd day.

Experiments made *in vitro* correspond with these results, and will be described later.

Experiments with Arsenophenylglycin.

Professor Ehrlich kindly sent some of this substance to Dr. Bagshawe, the Director of the Sleeping Sickness Bureau, with which we have made some initial experiments upon rats. Ehrlich found that Nagana mice could be cured, in practically every case, with this substance. But the effects on larger animals, so far as we have gone, are not quite so satisfactory, and it compares in this undesirable manner very well with the antimony tartrates, with which we can cure practically every case of Surra in rats, but which do not have anything like the corresponding effects on rabbits, guinea-pigs, and dogs. It is not only in the question of practical dosage that difficulties arise: each kind of animal has a personal equation, and their reaction to a given drug is not similar. This, and the relatively larger dosage in bigger animals, present considerable practical difficulties in the treatment of trypanosomiasis.

Our experiments have given the following results. Out of eight Surra rats of 180 to 200 grammes weight which were given one dose of 25 minims of a 1 in 80 solution of arsenophenylglycin, four died on the 19th day with living trypanosomes in their blood, the recurrences having taken place on the 16th—17th day. Two were given three and five doses respectively of 5 minims of a 1 per cent. solution of lithium antimonyl tartrate on the 17th and following days, and they lived 59 and 51 days. Of the two which are still living (95 days), one has had five doses of 5 minims of a 1 per cent. solution of lithium antimonyl tartrate, beginning on the 17th day, and the other had one similar dose given on the day before the recurrences occurred in the other rats.

The following experiment shows the effect of this substance upon the trypanosomes in the blood, and how much longer it takes than the antimony salts to produce its effects.

A Surra rat on the fourth day of the disease was treated with 1 c.c. of a 2 per cent. solution of arsenophenylglycin (practically the same dose as given to the other rats).

Blood was taken	and showed the following:
½ hour after injection...	Trypanosomes showed slight increase of motility.
1 ,, ,, ...	Ditto.
2 hours ,, ...	Ditto, but more marked.
3 ,, ,, ...	Trypanosomes not quite so active and fewer in number.
4 ,, ,, ...	Trypanosomes now very few in number.
4½ ,, ,, ...	Only one or two trypanosomes to be seen in a preparation.
5 ,, ,, ...	No trypanosomes seen.

In these specimens no swollen, breaking up, or "battledore" forms were seen: the trypanosomes simply disappeared.

On the Effects of the Drugs used upon the Trypanosomes in the Living Body.

In studying the therapeutic effect of the various drugs tried, including metallic antimony in a state of finest division, repeated observations of the peripheral blood were made in order to observe the effect of the drug upon the trypanosomes, and to ascertain when the trypanosomes entirely disappeared from the blood. The first stage noticed of the effect of the drug was a great increase in the motility of the trypanosomes, followed by a gradual slowing down to movements slower than normal. At this stage there is a tendency for the whole trypanosome to swell, and to become bloated in appearance. The swelling of the trypanosome continues until it becomes almost spherical in form, or oftener "battledore" shaped; the protoplasm becomes indistinct, and the flagellum appears to be attached to only one side of the periphery; the macro-nucleus is fairly distinct, but it eventually breaks up, and then the swollen mass disintegrates. The spleen at this time is full of these broken up masses of trypanosomes, and as the nuclei will still stain, a plasmodial appearance is seen in films of bits of nuclei dotted about in a granular ground. These stages can be observed after treatment with all the salts of antimony used, and are well marked after the administration of the metal, in which case, however, the stages are slower. The soluble salts, lithium and sodium antimonyl tartrates, effect the total disappearance of the trypanosomes in about one hour. Metallic antimony, when given in the various media tried (Lambkin's medium, olive oil, cod liver oil, heavy paraffin oil, egg-yolk), brings about this disappearance in from two-and-a-half to four hours, according to the medium used; the first noticeable effects being produced in about half an hour. In the case of egg-yolk and olive oil the blood is free from trypanosomes in two-and-a-half hours. This would seem to show that some portion of the metal introduced must be changed into some soluble form very rapidly; but, apparently, after the reaction of the tissues occurs the antimony becomes more or less shut off, and absorption must take place very slowly, as traces of the metal, apparently unaltered, have been found as late as six to seven weeks after the injection.

Sodium antimony lactate and antimony sodium calcium lactate were found to act rather more slowly than the above (see Table above), the time at which the trypanosomes had completely disappeared varying from three to four hours.

It was noticed in these experiments that trypanosomes, though obviously drug-affected when the blood was taken, remained alive on the slide outside the body for a long time after all forms had disappeared from the circulating blood.

Further details of the time taken for the various drugs to act will be found in the sections upon sodium antimony lactate, antimony oil, antimony egg-yolk, and arsenophenylglycin.

On the Action of Trypanocidal Substances in vitro.

Experiments have been carried out with a view of throwing light on the more exact nature of the changes which are produced in trypanosomes when they are brought into contact with trypanocidal substances. The general principles we have observed in these experiments have been:—1. To dissolve the drug in some fluid so that when it is added to the infected blood it will not cause osmosis to occur in the cellular elements of, or trypanosomes contained in the blood. (The various substances were dissolved in a 0·89 per cent. salt solution, isotonic with rat's blood which was used in these experiments.) 2. To use always equal volumes of the solution and of the affected blood. 3. To use blood at the time when the trypanosomes are just becoming very numerous, so as to avoid the presence of old, feebly moving forms, which are always present in the later stages of an acute infection. The method of observation has been to watch the behaviour of the trypanosomes when in contact with the various solutions of the drug under the microscope. A measured drop of blood and of the solution are mixed on a slide with care; the mixed drop is then covered with a sufficiently large cover glass, and this is sealed with vaseline.

It has been found possible in this manner to exactly determine the dilutions at which the various drugs used cease to have an instantaneously trypanocidal action; further, in higher dilutions, by carefully watching the changes taking place in the trypanosomes, it is possible to determine the dilution at which no effect is produced, and between these two points the periods of time necessary to ensure immobility and death of the trypanosomes can be ascertained. By a comparison of the results obtained a very good estimate of the probable action of any drug when given to an affected animal can be arrived at.

For instance, sodium and lithium antimonyl tartrates were found to act, in the same dilutions, in a manner fairly comparable to their antimony content, and to their action on the trypanosomes in an affected animal. Again, with atoxyl a much higher concentration of the drug was necessary—it had to be about ten times stronger—in order to obtain the same destruction pictures, results corresponding with the rapidity of the disappearance of trypanosomes from the peripheral blood of affected animals when treated with the above drugs.

In the case of the two new lactates mentioned above, their therapeutical value was accurately foretold by a preliminary study of their action *in vitro* in the manner described. In all these experiments controls have been carried out; it has been found that trypanosomes will live and retain their activity for hours when infected blood and the diluting fluid alone are mixed together.

The various changes taking place in trypanosomes on coming into contact with a dilute trypanocidal drug, commencing with their preliminary extraordinary increase of activity, and their subsequent swelling up, immobility and disintegration, can be watched in all their different stages in this manner. These effects resemble very closely the changes which take place in the trypanosomes in the peripheral circulation of an animal treated with antimony.

The following tables show the effects produced by the different substances in their various dilutions.

Dilutions of sodium antimonyl tartrate in 0·89 per cent. salt solution mixed with Surra rat's blood, in equal parts. The control in all cases is equal parts of blood and 0·89 per cent. salt solution.

Dilutions.				Time.	Control.
1—500.	1—1,000.	1—5,000.	1—10,000.		
Motionless	Motionless	Few active forms.	Trypanosomes active.	1 min.	Very active.
,,	,,	Motionless	Few active forms; rest sluggish.	10 mins.	,,
,,	,,	,,	All sluggish ...	30 ,,	,,
,,	,,	,,	Motionless ...	1 hour.	,,

Dilutions of lithium antimonyl tartrate in 0·89 per cent. salt solution mixed with Surra rat's blood, in equal parts.

Dilutions.					Time.	Control.
1—500.	1—1,000.	1—5,000.	1—10,000.	1—20,000.		
Motionless.	Motionless.	Some active trypanosomes.	Active trypanosomes.	Very active trypanosomes.	1 min.	Very active.
,,	,,	Motionless	Some active trypanosomes.	Many active trypanosomes.	10 mins.	,,
,,	,,	,,	Practically no motile trypanosomes seen, only 1 or 2 in a slide. Tendency to clump.	Few active trypanosomes seen. Tendency to clump.	30 ,,	,,
,,	,,	,,	Motionless ...	1 or 2 active forms seen. Rest motionless.	1 hour	,,

In a dilution experiment with lithium antimonyl tartrate made with the blood of a Surra rat after a second recurrence, after treatment with antimony (metal) and on first recurrence with lithium antimonyl tartrate, the trypanosomes *in vitro* appeared to have a greater resistance to the dilute drug than the stock strain.

A comparison of the following table with the previous one will demonstrate this:—

Dilutions.		Time.
1—1,000.	1—500.	
A few active forms present...	A number of active forms present ...	1 min.
Motionless	A few active forms seen	10 mins.
„	Motionless	30 „

This bears out our experience that the recurrences become less and less amenable to antimony as they increase in number.

The following table shows the action of atoxyl and lithium antimonyl tartrate compared in the above manner:—

Dilutions of Atoxyl.			Time.	Dilutions of Lithium Antimonyl Tartrate.		
1—500.	1—1,000.	1—5,000.		1—500.	1—5,000.	1—10,000.
Trypanosomes, all active.	Active ...	Active	1 min.	Trypanosomes, all motionless.	All markedly affected.	All fairly active.
Active, but affected.	„ ...	„	5 mins.	Motionless; commencing disintegration.	Motionless	Less active.
Less active ...	Sluggish, but still many active.	„	15 „	Only *débris* seen.	„	Some still moving; tendency to clump.
Practically motionless.	Nearly all motionless; 1 or 2 active forms seen.	Many moving still.	2 hrs.	„	Disintegrated.	Motionless; some disintegration.

Concentrated decoction of quassia in 0·89 per cent. salt solution mixed with Surra rat's blood in equal parts.

Dilutions.				Time.	Control.
1—500.	1—1,000.	1—5,000.	1—10,000.		
Very active...	Very active	Very active	Very active	1 min.	Very active.
„ ...	„	„	„	10 mins.	„
„ ...	„	„	„	30 „	„
„ ...	„	„	„	1 hour	„
Less active...	Less active	Less active	Less active	2 hours	Less active.

The conditions of the dilutions and the control were precisely similar at the end of two hours. There was no swelling nor clumping.

Dilutions of arsenophenylglycin in 0·89 per cent. salt solution mixed with Surra rat's blood in equal parts.

Dilutions.					Time.	Control.
1—100.	1—500.	1—1,000.	1—5,000.	1—10,000.		
Very active ...	Very active.	Very active.	Very active.	Very active.	1 min.	Very active.
Irritated: movements rapid and convulsive.	Activity increased.	,,	,,	,,	10 mins.	,,
Nearly motionless.	Sluggish	,,	,,	,,	30 ,,	,,
Motionless ...	,,	,,	,,	,,	1 hour	,,

Experiments in vitro *performed with the Blood of a Normal Rat which had been treated with Antimony.*

Experiments were made in order to ascertain whether the blood of a rat which had been treated with antimony would show any active trypanocidal powers *in vitro*. Although in the case of an infected animal all the trypanosomes in the peripheral blood would have been destroyed in about an hour, no noticeable trypanocidal effects were shown by the blood of a treated rat in the following experiments.

A normal rat had 5 minims of a 1 per cent. solution of lithium antimonyl tartrate injected subcutaneously; its blood was taken at 15, 30, 60, and 70 minutes after the injection, and was mixed with an equal quantity of blood from a Surra rat containing many trypanosomes; the mixed bloods, taken at the times mentioned, were examined under the microscope at various intervals from 5 to 30 minutes after the mixing, and the trypanosomes were found to be entirely unaffected, so that the blood of the treated normal rat did not have any trypanocidal effect added to it by the dose of lithium antimonyl tartrate. The Surra rat, whose blood was used for this experiment, was then given 5 minims of a 1 per cent. solution of lithium antimonyl tartrate:—

Blood was taken	and showed the following:
10 minutes after infection ...	Trypanosomes affected: movement very rapid.
20 ,, ,, ...	Many "battledores."
40 ,, ,, ...	Trypanosomes greatly decreased in number all "battledores."
60 ,, ,, ...	Blood quite free from trypanosomes.

A normal rat was given four doses subcutaneously, one every other day, of 5 minims of a 1 per cent. solution of lithium antimonyl tartrate; 24 hours after the last dose a drop of its blood was mixed with a drop of blood from a Surra rat in which trypanosomes were plentiful. The mixture was watched under the microscope for half an hour, but no effect was produced; the blood of the treated animal behaving just as the blood of the control, an untreated rat.

A normal rate was given subcutaneously 10 minims (a lethal dose) of a 1 per cent. solution of lithium antimonyl tartrate, and its blood was mixed at half an hour, one hour, and one-and-a-half hours after the injection with an equal part of an emulsion of trypanosomes prepared from the lungs, liver, and heart's blood of a Surra rat just dead. Each of the mixtures was examined up to 30 minutes, but no effect whatever was produced on the trypanosomes. These experiments may be compared with those recorded on p. .

Experiments with Antimony upon Dogs.

Since the date of the last paper a large number of experiments have been made with antimony in various forms upon dogs suffering from Surra. Of the five dogs mentioned there, one remains alive and well at the present date, more than a year after inoculation.

Our experiences with dogs show that they are extremely susceptible both to the disease and also to antimony: they are therefore not quite suitable animals for these experiments, although they have all lived many times the length of the untreated disease, that is 14 days. Five of the dogs were treated with small doses of sodium antimonyl tartrate in their drinking water, but the disease is so acute in dogs that this method of giving the drug, although it appeared to have some effect in postponing the reappearance of the trypanosomes in the blood, did not produce results sufficiently encouraging to warrant further experiments.

With regard to the experiments made with metallic antimony suspended in egg-yolk, the initial experiment was so encouraging as to make a further trial necessary. In this case the dog at the first relapse was given 20 minims of a 2½ per cent. suspension: there was no local reaction, which in dogs is of frequent occurrence after the administration of antimony in any form, and the trypanosomes, which were very numerous, were entirely absent from the blood in 24 hours; the dog remained quite free from them for 48 days, and gained 3 lbs. in weight, and appeared perfectly well. The recurrence was very sudden, as the dog was perfectly well up to the moment when he was seized with a series of fits which ushered in the recurrence, from which he did not recover. A rat treated at the same time as this dog with 5 minims of the same suspension is alive and well more than 100 days after this one dose.

Many of the dogs mentioned in the table below have died with fits and paralyses and other nervous symptoms, but we are not certain whether these are due to the disease or to the antimony. In certain of the dogs the treatment has appeared to alter the acute disease into a chronic one, and in one of these more chronic cases there was a considerable excess of cerebro-spinal fluid and a cellular exudation around the vessels in the brain, very similar in incidence and extent to that described and figured by one of us in rats dead from infection with *Trypanosoma gambiense*.*

There is a curious uncertainty in the local effects produced in dogs by antimony, whether injected subcutaneously or intramuscularly, and they vary from time to time in the same dog; sometimes little or no effect is produced, and sometimes the suppuration and necrosis produced are sufficient to kill the animal.

We have recently given 24 injections of lithium antimonyl tartrate subcutaneously to three dogs in the greatest possible dilution; of these three places have suppurated slightly, although the conditions under which they were given were similar to those under which the 21 other doses were given. (These dogs are now living and well 53 days after inoculation, and they have had no recurrences.)

The following table gives a synopsis of the treatment, &c., of Surra dogs:—

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* 'Roy. Soc. Proc.,' B, vol. 79, p. 95.

Average Duration of Untreated Disease, 14 days.

No.	Weight, in Kilos.	Number of Doses.	Quantity of Dose, in Minims.	Material.	Recur- rences.	Remarks.
1	11	2	20	5 per cent. ant. cream	2	*Dog is alive and well 373 days after inoculation.*
		2	20	2 ,, sod. ant. tart.		
2	11	4	20	2 ,, ,, ,, ...	3	Died on 94th day : no trypanosomes found for 21 days before death. There were 41 days between the first and second recurrences. Died with fits and nervous symptoms.
		8	20	2 ,, lith. ant. tart.		
3	18¾	4	20	5 ,, sod. ant. tart. cream	6	Died on 67th day. No trypanosomes found for 22 days before death. Died with fits and nervous symptoms.
		2	20	2 ,, ,, ,,		
		1	20	5 ,, ant. cream.		
		4	20	2 ,, lith. ant. tart.		
4	8	3	12	5 ,, sod. ant. tart. cream	4	Died of distemper on 63rd day. No trypanosomes found for 7 days before death.
		1	20	2 ,, ,, ,,		
		7	12	2 ,, lith. ,,		
5	6¾	3	10	5 ,, sod. ,, cream	3	Died of pneumonia on the 53rd day. No trypanosomes found for 11 days before death.
		2	20	1 ,, lith. ,,		
		3	10	2 ,, ,, ,,		
6	6¼	5	10	2 ,, ,, ,, ...	1	Died from abscess on the 40th day. No trypanosomes found for 17 days before death.
		4	20	2 ,, ,, ,,		
7	7¼	5	10	2 ,, ,, ,, ...	3	Died from abscess on the 61st day. No trypanosomes in blood for 10 days before death.
		7	20	2 ,, ,, ,,		
8	14½	4	20	2 ,, ,, ,, ...	2	Died on 55th day with fits and nervous symptoms. Trypanosomes in blood. Antimony given in water also.
		2	12	5 ,, ant. oil.		
9	12¾	2	15	2 ,, lith. ant. tart ...	2	Died on 77th day from abscess. No trypanosomes seen for 16 days before death. Antimony given in water also.
		2	15	5 ,, ant. oil.		
		1	20	5 ,, ,,		

No.	Weight, in Kilos.	Number of Doses.	Quantity of Dose, in Minims.	Material.	Recurrences.	Remarks.
10	13¼	2	12	5 per cent. ant. oil.	3	Died on 63rd day with nervous symptoms and paralysis. No trypanosomes found. Antimony given in water also.
		1	20	2 ,, lith. ant. tart.		
		2	15	2 ,, ,, ,,		
11	8½	3	20	1 ,, ant. sod. lact.	4	Died on the 66th day with nervous symptoms. No trypanosomes found after death. Antimony given in water also.
		1	15	2 ,, lith. ant. tart.		
		1	10	5 ,, ant. oil.		
		1	15	5 ,, ,,		
		1	5	2½ ,, ,,		
		2	15	2½ ,, ,,		
12	10	1	20	1 ,, ,,	3	Died on the 65th day with living trypanosomes in blood. Antimony given in water also.
		2	10	5 ,, ,,		
		2	15	5 ,, ,,		
		4	15	2 ,, lith. ant. tart.		
13	11½	1	20	2 ,, ,, ,,	1	Died on the 60th day with nervous symptoms. No trypanosomes seen for 29 days before death.
		1	15	2½ ,, ant. oil.		
		2	15	5 ,, ,,		
		1	20	5 ,, ,,		
14	10½	2	15	5 ,, ,,	2	Died on the 52nd day from abscess. No trypanosomes found after death.
		1	20	5 ,, ,,		
		1	15	2 ,, lith. ant. tart.		
		2	20	2 ,, ,, ,,		
15	13¾	1	15	5 ,, ant. oil	5	Died on the 50th day with living trypanosomes in blood.
		2	20	5 ,, ,,		
		3	20	2 ,, lith. ant. tart.		
16	9¾	1	15	2 ,, ,, ,,	0	Died on the 64th day from abscess. No trypanosomes found after death and no recurrences.
		2	15	5 ,, ant. oil.		
		1	10	2½ ,, egg ant.		
17	9¼	2	15	5 ,, ant. oil	0	Died on the 48th day from pneumonia. No trypanosomes found after death.
		1	15	2 ,, lith. ant. tart.		

18	9¼	1	15	2 ,,	,, ,,	1	Died on 50th day, possibly from ant. sod. lactate. No trypanosomes found after death.
		2	15	5 ,,	ant. oil.		
		1	20	1 ,,	ant. sod. lact.		
19	10¼	1	20	2 ,,	lith. ant. tart.	3	Died on 74th day with nervous symptoms. There were 48 days between the first and second recurrences.
		1	15	5 ,,	,, ,,		
		1	10	5 ,,	,, ,,		
		1	20	5 ,,	ant. cream.		
		1	20	2½ ,,	egg ant.		
		1	10	2½ ,,	,,		
20	7¼	3	15	2½ ,,	,,	—	Trypanosomes practically never out of blood. Died on the 37th day, paralysed.
		1	15	5 ,,	,,		
21	13½	2	10	2½ ,,	,,	—	Trypanosomes practically never out of blood. Died on the 44th day with fits and nervous symptoms.
		3	20	5 ,,	,,		
		1	15	2 ,,	lith. ant. tart.		
22	8½	3	15	2½ ,,	egg ant	—	Trypanosomes practically never out of blood. Died on the 55th day with fits and nervous symptoms.
		3	15	2 ,,	lith. ant. tart.		
23	13¼	1	15	5 ,,	egg ant.	3	Died on the 55th day with fits and nervous symptoms.
		2	20	5 ,,	,,		
		2	15	5 ,,	lith. ant. tart.		
		4	5	5 ,,	,, ,,		
24	12¼	1	15	5 ,,	egg ant.	2	Died on the 64th day with living trypanosomes in the blood.
		1	20	5 ,,	,,		
		1	15	5 ,,	lith. ant. tart.		
		1	10	5 ,,	,, ,,		
		5	5	5 ,,	,, ,,		
25	9¾	1	10	5 ,,	,, ,,	2	Died on the 56th day with nervous symptoms.
		1	20	5 ,,	egg ant.		
		5	5	5 ,,	lith. ant. tart.		
26	8	1	10	5 ,,	,, ,,	2	Died on the 47th day. Trypanosomes found in the cerebro-spinal fluid.
		1	20	5 ,,	egg ant.		
		5	5	5 ,,	lith. ant. tart.		

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33. THE DEVELOPMENT OF TRYPANOSOMA GAMBIENSE IN GLOSSINA PALPALIS.

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[PLATES 4 AND 5.]

The following experiment is so complete in itself that no apology is offered for publishing it by itself. In 1903 the Sleeping Sickness Commission of the Royal Society came to the conclusion that the carrying of infection from a sleeping sickness patient to a healthy person by the *Glossina palpalis* was a mechanical act, and required no previous development of the parasite within the fly. The Commission also held that the power of transferring the disease was lost to the fly 48 hours after it had fed on an infected person.

Koch and Stuhlmann, in German East Africa, described developing forms in *Glossina*, but did not succeed in infecting healthy animals by the injection of these forms.

Kleine, in German East Africa, at the end of 1908, succeeded first in showing that *Glossina palpalis* could convey *Trypanosoma brucei* some 50 days after the fly had fed on an infected animal.

It seems, at first, strange that this fact should have escaped notice for 15 years, and can only be accounted for by assuming that it is an event of the rarest for a fly to be found which fulfils the unknown conditions necessary for the development of the trypanosomes in its interior. If we assume that it is only one fly in a hundred or in a thousand in which this development takes place, then the difficulty of observing the phenomenon can be understood.

Take the following experiments, for example:—

Table I.—Flies caught in an Infected Area, kept for some days, and then fed on Healthy Animals.

Trypanosoma brucei—Glossina morsitans.

Expt.	Place.	Observer.	No. of Flies fed.	No. of times Flies fed.	No. of Days before Infection or under observation.	Result.
210	Zululand ...	Bruce ...	5	32	64	Negative.
242	" ...	" ...	30	11	56	"
232A	" ...	" ...	50	15	34	"

These experiments seemed to show that if flies caught in a highly infected district, into which a horse could not be taken even for a few hours without contracting nagana, are kept without food for a few days—say three to five—they are then incapable of conveying infection. This appeared to be a strong proof that the duration of infectivity in the fly was a short one, since, if this were not the case, 1 of the 85 flies ought to have been in a condition capable of infecting, having, of course, been infected at some previous date in the "fly country." It may be repeated, that these flies were caught in a most highly infected district, so that if *Glossina morsitans* can remain infective for 50 or 60 days, 1 at least of the 85 ought to have been in the condition which made it capable of conveying the disease.

This development of the trypanosomes in the fly is strikingly like what occurs in the test-tube with Novy's medium. A thousand tubes are inoculated with *Trypanosoma brucei*: the trypanosomes all appear to die off, but 20 days afterwards a peculiarly resistant individual is found in one tube of the thousand, who has adapted himself to the new environment, and soon multiplies into myriads. What it is which enables this particular individual to adapt itself to such altered conditions is unknown. It is the merest speculation to call it a sexual act and pick stout forms as females and slender forms as males.

Again, because this late development of the trypanosomes enables a particular fly to remain infected for 100 days, or even possibly for the remainder of its life, it by no means follows that this is the usual method of infection. The mechanical transference of the disease is proved up to the hilt, and for every case which falls a victim to the rare late-infected fly, a thousand must be infected by direct mechanical transference.

SUMMARY OF THE EXPERIMENT WHICH FORMS THE SUBJECT OF THIS PAPER.

Before describing at length the experiment which forms the subject of this paper, we may summarise it as follows:—

1. On March 5, 1909, 60 *Glossina palpalis* caught on the lake shore were placed in two cages, 30 in each. The flies were fed on two infected monkeys for 2 days. They were then starved for 72 hours to get rid of mechanical transference. The following 5 days they were placed on a healthy monkey, and every successive period of 5 days, or thereabouts, on a fresh monkey, up to 86 days, when the experiment came to an end. The result was, that the first two monkeys remained healthy, but that all the following monkeys, up to 75 days, became infected with *Trypanosoma gambiense*.

2. If 7 days be deducted for the incubation period, then the flies first became infected 18 days after their first feed on an infected animal.

3. There is some evidence that among the 60 flies only 1 was infective. Fifty-four days after the beginning of the experiment each cage was placed on a separate monkey. Up to that time both the cages of flies had been fed on the same animal. Cage A contained, after 54 days, 11 flies. Cage B, 4 flies. Cage A continued to infect monkeys for 21 days more, making a total of 75 days. Cage B did not infect. Again, as was natural, the flies gradually died off during the experiment, and as each fly died it was carefully dissected and examined for trypanosomes. Not a single trypanosome of any kind whatever was seen in any dissected fly up to 75 days, when a fly died in Cage A which was found to be swarming with trypanosomes similar to *Trypanosoma gambiense*. After the death of this fly, Cage A ceased to be infective, and when the experiment was stopped the remaining flies were killed off and dissected, but among them not a sign of a trypanosome could be seen. In the same way the flies remaining in the non-infective Cage B were examined, with a similar negative result.

4. Here follows an interesting and unique observation. A tiny drop of fluid taken from the gut of the 75-day fly injected under the skin of a monkey gave rise to Sleeping Sickness after an incubation period of eight days. This, so far as we are aware, is the first time this has been recorded.

5. It will be seen from the detailed experiment that the flies were starved for three days between several of the experiments. This, of course, was to get rid of the fallacy of mechanical transference.

6. It may be said that perhaps these monkeys became infected by some other means than the flies in the cage—for example, by other biting flies, or by contact. To this it may be answered that there are more than 200 monkeys under observation here, sick and healthy. They are all examined twice a week, but during the last eight months not a single case of accidental infection has taken place.

DETAILS OF THE EXPERIMENT.

Experiment 663.

To ascertain if development of *Trypanosoma gambiense* takes place in the interior of *Glossina palpalis*, and if so, how long does the fly remain infective.

March 5, 1909.—Two batches of *Glossina palpalis* caught on the Lake shore, consisting of 30 flies in each batch, were fed on monkeys. Experiments 568 and 214, whose blood contained numbers of *Trypanosoma gambiense*.

March 6.—The flies again fed as on the 5th, to ensure that as many as possible should get a feed of the infected blood. Nearly all the flies fed on one or other occasion. The flies are kept in a moist atmosphere at 22° C.

The following table gives the principal details of the experiment:—

Table II.

Date.	Day of Experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Mar. 5	—	Flies fed on infected monkey.			
6	1	" " "			
7	2	Flies starved 72 hours.			
8	3	" "			
9	4	Fed on Monkey 579 ...		–	
10	5				
11	6				
12	7				
13	8				
14	9	" " 651 ...		–	
15	10				
16	11				
17	12				
18	13				
19	14	" " 652 ...	+		
20	15				
21	16				
22	17				
23	18				
24	19	" " 653 ...	+		
25	20				
26	21				
27	22				
28	23				
29	24	" " 654 ...	+		
30	25				
31	26				
Apr. 1	27				
2	28				
3	29	" " 655 ...	+		
4	30				
5	31				
6	32				
7	33				
8	34	" " 672 ...	+		
9	35				
10	36				
11	37				
12	38				
13	39	" " 722 ...	+		
14	40				
15	41				
16	42	Starved for 72 hours.			
17	43				
18	44	Fed on Monkey 727 ...	+		
19	45				
20	46				
21	47				
22	48				
23	49				
24	50				
25	51				

Date.	Day of Experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
Apr. 26	52	Starved for 76 hours.			
27	53				
28	54	Cage A fed on Monkey 735	+		
29	55				
30	56				
May 1	57				
2	58	„ B „ „ 736		—	
3	59	Starved for 74 hours.			
4	60				
5	61	Cage A fed on Monkey 749	+		
6	62				
7	63				
8	64				
9	65	„ B „ „ 748		—	
10	66	Starved for 72 hours.			
11	67				
12	68	Cage A fed on Monkey 765	+		
13	69				May 13.—Flies remaining in Cage B killed and dissected.
14	70				
15	71				
16	72	„ B „ „ 764		—	
17	73	Starved for 72 hours.			
18	74				
19	75	Cage A fed on Monkey 848		—	May 19.—Fly 866 found dead in Cage A and dissected. Did not feed on Monkey 848.
20	76				
21	77				
22	78				
23	79				
24	80	Starved for 72 hours.			
25	81				
26	82	Cage A fed on Monkey 911		—	Expts. 848 and 911 healthy on 7th June, 1909.
27	83				
28	84				
29	85				
30	86				
31	87	Experiment stopped.			Remaining flies killed and dissected.

Remarks on the Experiment.

Everyone will agree that this is a most interesting experiment. It is evident that a single infected fly did all the mischief, and by good luck this fly was detected. Captain A. E. Hamerton. D.S.O., had charge of the experiment at first, and on his leaving Mpumu about the beginning of May, it fell to Sergeant A. Gibbons, Royal Army Medical Corps. Both are to be congratulated on the results, which are the outcome of care and thoroughness. Captain F. P. Mackie had the good fortune to dissect the fly which did the injury, and which will be fully described later.

INCUBATION PERIOD.

From the experiment may be drawn the incubation period in monkeys bitten by a late-infected fly.

It is remarkable how regular this is in those monkeys which gave a positive result. This shows how very infective Fly 866 was. Apparently each time it bit it infected.

The following table gives the period of incubation in each case:—

Table III.

Date.		Experiment.	Flies first fed.		Trypanosomes appeared in Blood.		Number of Days before Trypanosomes appeared in Blood.
1909.			1909.		1909.		
March 19	...	652	March 19	...	March 30	...	11
,, 24	...	653	,, 24	...	April 2	...	9
,, 29	...	654	,, 29	...	,, 6	...	8
April 3	...	655	April 3	...	,, 13	...	10
,, 8	...	672	,, 8	...	,, 15	...	7
,, 13	...	722	,, 13	...	,, 20	...	7
,, 18	...	727	,, 18	...	,, 24	...	6
,, 28	...	735	,, 28	...	May 5	...	7
May 5	...	749	May 5	...	,, 11	...	6
,, 12	...	765	,, 12	...	,, 17	...	5

Leaving out the first experiment, 652, as it is doubtful as to the exact day Fly 866 became infective, this gives an average incubation period of seven days. It would therefore appear that Fly 866 probably infected each animal on the first day it bit it, showing how dangerous such an infected fly is.

Description of the *Glossina palpalis*, Fly 866, which was dissected 75 Days after having fed on a Monkey whose Blood contained *Trypanosoma gambiense*.

Experiment 866.

May 19, 1909.—Dissected a *Glossina palpalis*, which was found dead to-day in Cage A of Experiment 663. On removing the viscera by the usual method, the mid-gut was seen to be of a pale salmon-pink. A small quantity of its contents, examined in the fresh condition, was found to contain enormous numbers of trypanosomes. The tube of this part of the intestine was absolutely crammed with active, seething masses of these flagellates. In regard to the other parts of the fly, nothing was seen in the proboscis. In the proventriculus one trypanosome only was found. The salivary glands contained large numbers of altered-looking trypanosomes, the fore-gut many large stout forms, with bright granules. The crop was empty and showed nothing. The Malpighian tubules, hind-gut, and proctodæum also were drawn blank.

In addition to examining these organs in the fresh condition, smears were made and stained. The examination of these stained specimens gave the following results:—

The salivary glands.—These had been carefully removed before the intestine was opened, and therefore had no chance of being fouled. As will be seen from the coloured drawing (Plate 4, fig. 1), the trypanosomes found in these glands differed from those seen in the intestine. The bodies are very irregular in shape, and contain, besides a reddish-stained nucleus, dark deeply-stained coarse chromatin granules. The other cell contents remain unstained. Free chromatin granules and flagella are to be seen scattered over the field. Sometimes the bodies are definitely pear-shaped, with a flagellum coming from the narrow end, and rarely a more definite trypanosome shape can be seen; but never a true trypanosome.

[It is a matter of deep regret that an inoculation experiment was not made with an emulsion of part of the salivary glands.]

The fore-gut.—The fore-gut contained many trypanosomes. The cytoplasm stains a pale blue, and the nucleus a reddish-purple. The micronucleus is not distinctly seen in some of the trypanosomes, but when it is, it is always distinctly posterior to the nucleus. The protoplasm contains many coarse darkly-stained chromatin granules. The undulating membrane is less marked than in the normal blood trypanosome, and the flagellum, which usually springs from a micronucleus-like body, is less deeply stained (Plate 4, figs. 6-13).

The mid-gut.—The mid-gut contained innumerable trypanosomes of the *gambiense* type. Some are dividing, and all have a well-marked nucleus and micronucleus, the latter at or near the posterior extremity. The protoplasm contains many chromatin granules, and an undulating membrane and flagellum are present (Plate 4, figs. 6-16). Many groups, or rosettes, composed of 15 to 20 individuals, occur, the flagella pointing outwards (Plate 5, fig. 1).

The *proboscis, proventriculus, thoracic gut, crop, hind-gut,* and *Malpighian tubes* contained no trypanosomes.

The most interesting thing in this description of the examination of Fly 866 is the condition of the salivary glands. How these trypanosome-like bodies, or derivatives of trypanosomes, got into them is a mystery, and we will content ourselves at present with merely placing the bare fact on record until the salivary glands of similarly infected flies are examined.

There is one fallacy which might be pointed out. It is assumed that Fly 866 became infected on the first or second day of the experiment. It is possible that it became infected when feeding on the fifth day on an animal which showed trypanosomes in its blood a day or two later. This, however, is unlikely, as no other fly showed trypanosomes on dissection.

In order to make the story more complete, on Plate 4, figs. 1-5, is represented the *Trypanosoma gambiense* from the blood of one of the monkeys on which the flies were fed at the beginning of the experiment, and on Plate 5, figs. 2-5, are shown *Trypanosoma gambiense* from the monkey which became infected from the contents of the mid-gut of Fly 866.

PROPORTION OF INFECTED FLIES TO NON-INFECTED IN NATURE.

In the experiment under consideration it is seen that, in artificially-infected flies, only 1 in 60 showed the phenomenon of late infectivity. In nature the proportion must be less, as many of the flies, in many places at least, can never have fed on an animal whose blood contained *Trypanosoma gambiense*.

That there can be but few under natural conditions Table IV. shows. The table is made by subtracting the flies fed on the animal during the last seven days, before trypanosomes were found in the blood, this being the incubation period, from the total number. The experiments consist in catching tsetse flies in the infected area, bringing them to the laboratory and placing them straightway on healthy animals.

The first two experiments were made with *Trypanosoma brucei* and *Glossina morsitans*, and it would appear from them that 104 and 108 flies were used respectively before an infective one was found. This perhaps explains why Bruce's 85 flies failed to infect.

Table IV.—Table to show Probable Number of Naturally Infected Flies per thousand.

Expt.	Place.	Observer.	No. of Flies fed before Infection took place.	Result.		Probable No. of Naturally Infected Flies per thousand.
				Positive.	Negative.	
TRYPANOSOMA BRUCEI—GLOSSINA MORSITANS.						
225	Zululand	Bruce	104	+		9·6
236	"	"	108	+		9·2
TRYPANOSOMA GAMBIENSE—GLOSSINA PALPALIS.						
94	Uganda ...	Bruce and Nabarro	89	+		11·2
130	" ...	Bruce, Nabarro, and Greig.	850	+		1·2
131	" ...	" " "	506	+		1·9
136	" ...	Nabarro and Greig	723		—	
228	" ...	Greig and Grey ...	866	+		1·2
301	" ...	" " ...	2,299		—	
45	Leopoldville.	Dutton, Todd, and Hannington.	457		—	
46	"	" " "	552		—	
128A	River ...	" " "	25		—	
139	" ...	" " "	262		—	
141	" ...	" " "	52		—	
182	Kasongo	" " "	211		—	
198	"	" " "	2,659	+		0·4
203	"	" " "	1,789		—	
213	"	" " "	717		—	
52	Uganda ...	Bruce, Hamerton, Bateman, and Mackie.	41		—	
214	" ...	" " "	3,284	+		0·3
568	" ...	" " "	178	+		5·6
571	" ...	" " "	850	+		1·2
53°	" ...	" " "	21		—	
612	" ...	" " "	615	+		1·6
674	" ...	" " "	2,315	+		0·4

° Animal died.

In the experiments with *Trypanosoma gambiense* and *Glossina palpalis* the average is 2·5 per thousand. It is, of course, impossible to tell how many of these positive experiments were infected by mechanical transference or by a late-infective fly; but, in any case, the proportion is small. If this were not so, all the native population of the Lake shore, and most of the Europeans in Uganda, would long ago have been blotted out.

DESCRIPTION OF PLATES.

PLATE 4.

Smear preparation of salivary glands of *Glossina palpalis*, Experiment 866, stained Giemsa, showing irregularly shaped trypanosomes, with unstained protoplasm, reddish-coloured nuclei, and deeply stained chromatin granules. Note the chromatin granules scattered singly about the field, each surrounded by a pale area, fig. 1. × 2000.

Normal *Trypanosoma gambiense* from monkey, Experiment 568, on which the flies were fed at the beginning of the experiment, figs. 2, 3, 4, and 5. × 2000

Trypanosomes from the mid-gut of infected fly, Experiment 866, figs. 6–16. × 2000.

PLATE 5.

Rosette form from the mid-gut, fig. 1. × 2000.

Trypanosoma gambiense from the blood of monkey, Experiment 868, into which a tiny drop of the contents of the mid-gut of Fly 866 had been injected, figs. 2–5. × 2000.

Trypanosomes from the fore-gut of Fly 866, stained Giemsa, figs. 6–13. × 2000.

[*Reprinted from the* PROCEEDINGS OF THE ROYAL SOCIETY, B. Vol. 81.]

34. A NOTE ON THE OCCURRENCE OF A TRYPANOSOME IN THE AFRICAN ELEPHANT.

By Colonel Sir DAVID BRUCE, C.B., F.R.S., Army Medical Service; Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, Royal Army Medical Corps; and Captain F. P. MACKIE, Indian Medical Service. (Sleeping Sickness Commission of the Royal Society, 1908.)

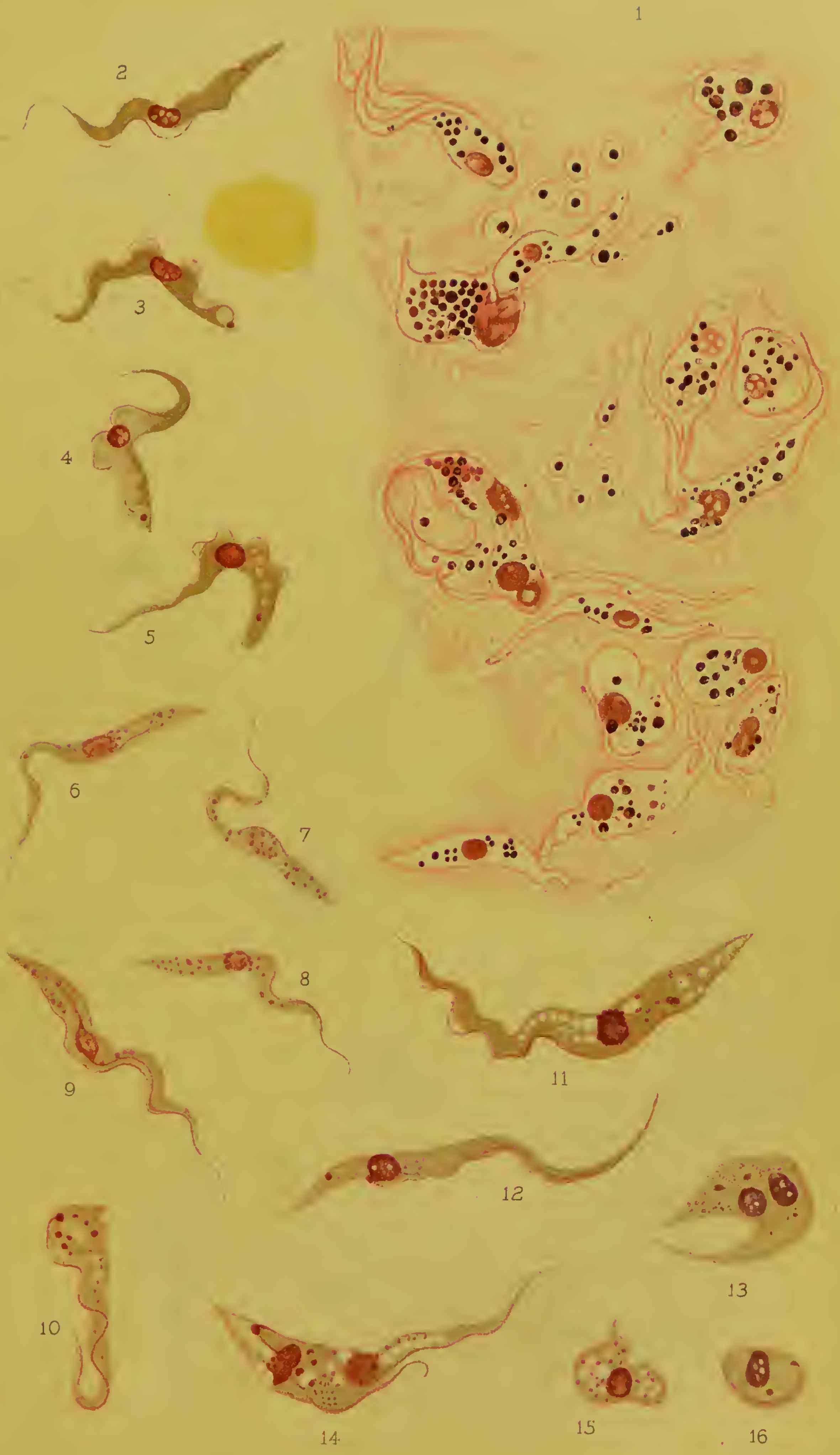
(Received July 5, 1909.)

[PLATE 6.]

As trypanosomes have never been reported as having been observed in the blood of the African Elephant, the Commission thought it would be of interest to note this observation.

In Laveran and Mesnil's book on trypanosomes, translated by Nabarro, on p. 261 it is stated that "the occurrence of Surra (*Trypanosoma evansi*) in elephants in India and Burmah is practically proved. In this connection we have only the statement of G. H. Evans that, in 1893, 14 out of 32 elephants died of the disease in Burmah." The year 1893 is almost prehistoric for trypanosomes. At that time observers have even failed to distinguish between the common rat trypanosome—*Trypanosoma lewisi*—and that of Surra. It may well be, then, that Evans was mistaken in his diagnosis of the species causing this large mortality in elephants.

The African elephant, in whose blood this trypanosome was found, was shot by Mr. L. C. Lea-Wilson, of the Uganda Company, Limited, at a spot two miles from the eastern shore of Lake Albert, near Ngogole, about 31° 10′ E. lat. and 1° 30′ N. long.



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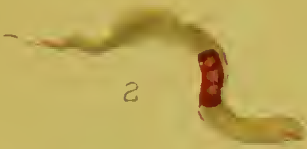
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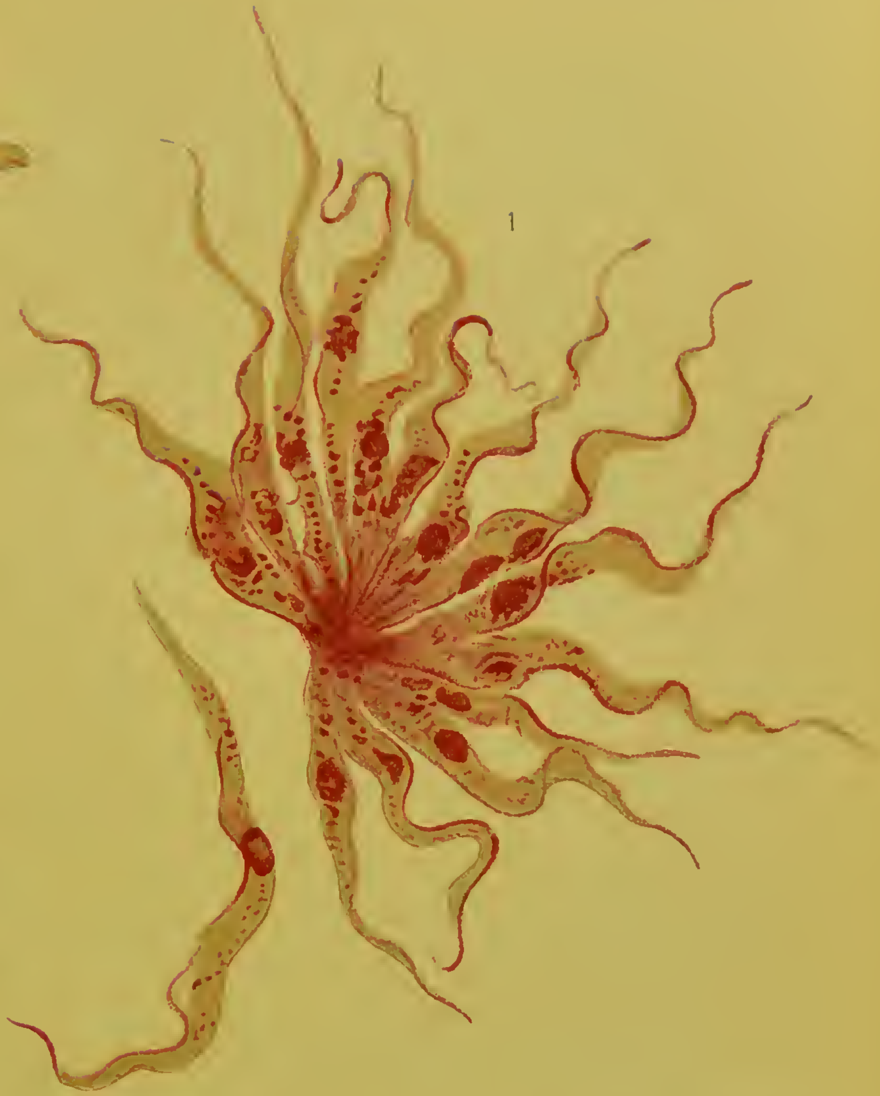
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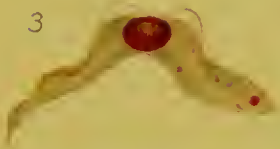
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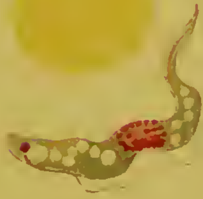
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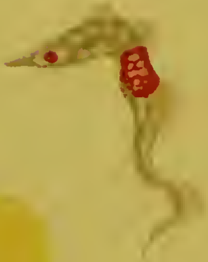
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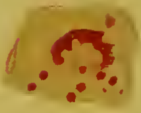
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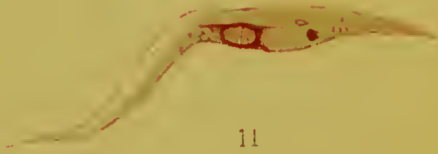
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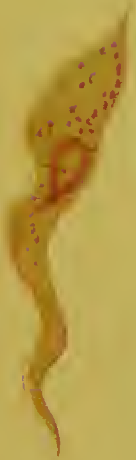
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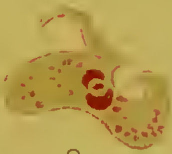
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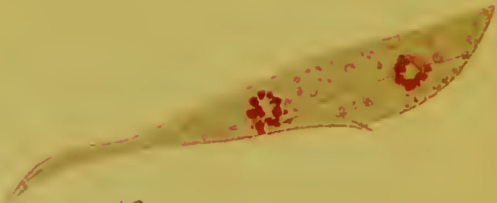
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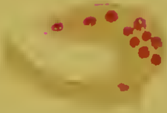
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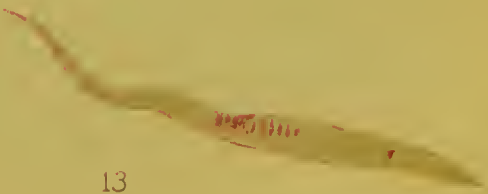
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It is to be regretted that none of the blood was injected into a dog, donkey, or ox, in order that a fuller study of this trypanosome might have been made. As it is, all the material available are a couple of smears made by Mr. Lea-Wilson and sent to the Commission.

Morphology of the Trypanosome of the Elephant.

Method of Fixing and Staining.—The two slides received from Mr. Lea-Wilson were fixed in osmic acid vapour and alcohol, stained in Giemsa, and decolorised in orange tannin.*

Length.—For method of measurement see the same paper, p. 16. As will be seen from the coloured plate, which was drawn by Sergeant Gibbons, R.A.M.C., this trypanosome is of medium size. The average length of 18 individuals is 18·5 microns: maximum 21, minimum 15.

Breadth.—On an average the breadth at the thickest part is 3 microns.

Shape.—This trypanosome is of the *Trypanosoma brucei* type, inasmuch as it has a well-developed undulatory membrane and free flagellum. As will be seen from the drawing (Plate 6), one noteworthy feature it has is the uniformity in size and shape of the different individuals. The posterior end is blunt, or conical, reminding one somewhat of the head of a seal, with the bulging micronucleus for an eye. The body thickens as far as the middle, when it gradually tapers away to the anterior end.

Contents of Cell.—The protoplasm is clear and particularly free from granules.

Nucleus.—The nucleus is compact and sharply defined from the neighbouring protoplasm. In shape it is round, or oval, and often lies nearer the anterior extremity than the posterior. Its length averages 2 microns.

Micronucleus.—The micronucleus is small, round, and distinct. It is situated close to the posterior extremity, and often appears to bulge above the surface.

Undulating Membrane.—The undulating membrane is well developed and thrown into well-marked folds.

Flagellum.—The flagellum stains deeply. It runs from the micronucleus along the edge of the undulating membrane, beyond which it projects as a free flagellum for some 5 or 6 microns.

Conclusions.

In our present state of knowledge it seems impossible to name trypanosomes from their form alone. We were, however, much surprised, a short time ago, by Sir John McFadyean separating with ease *Trypanosoma brucei* from *Trypanosoma evansi.* If this can be done for such closely related species, surely it should be possible to do it for all. To assist to this end it would be well if observers would adopt one method of fixing, staining, and measuring. In the "Third Report of the Wellcome Research Laboratories," Khartoum, facing p. 30, there is a coloured plate of trypanosomes, stated to have a magnification of 1000. On measuring one of them it is found to have a magnification of between 2000 and 3000. Then, again, many of the trypanosomes depicted are dividing forms, which is misleading.

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* *Vide* 'Roy. Soc. Proc.,' Series B, vol. 81, p. 16.

The method of measuring must also make a difference. For example, in Laveran and Mesnil's book the length of *Trypanosoma brucei* in the rat is given as 26 to 27 microns, whereas by our method of measuring the average length of 20 individuals is 22·8 microns: maximum 25, minimum 20.

The trypanosome of the elephant has an average length of 18·5 microns: maximum 21, minimum 15, a well-developed undulatory membrane and free flagellum. The trypanosomes with free flagella are *Trypanosoma brucei, cazalboui, evansi, gambiense, pecaudi,* and *soudanense.* It probably is neither *Trypanosoma cazalboui* nor *pecaudi,* on account of its well-developed undulating membrane and uniform size. Under the circumstances it is impossible to decide as to its identity with *Trypanosoma brucei, gambiense,* or *soudanense,* but if a guess were hazarded then it would be *Trypanosoma soudanense.*

Until the nature of this species is better known we propose to name it *Trypanosoma elephantis.*

[*Reprinted from the* PROCEEDINGS OF THE ROYAL SOCIETY,
B. Vol. 82.]

35. SLEEPING SICKNESS IN UGANDA.—DURATION OF THE INFECTIVITY OF THE GLOSSINA PALPALIS AFTER THE REMOVAL OF THE LAKE-SHORE POPULATION.

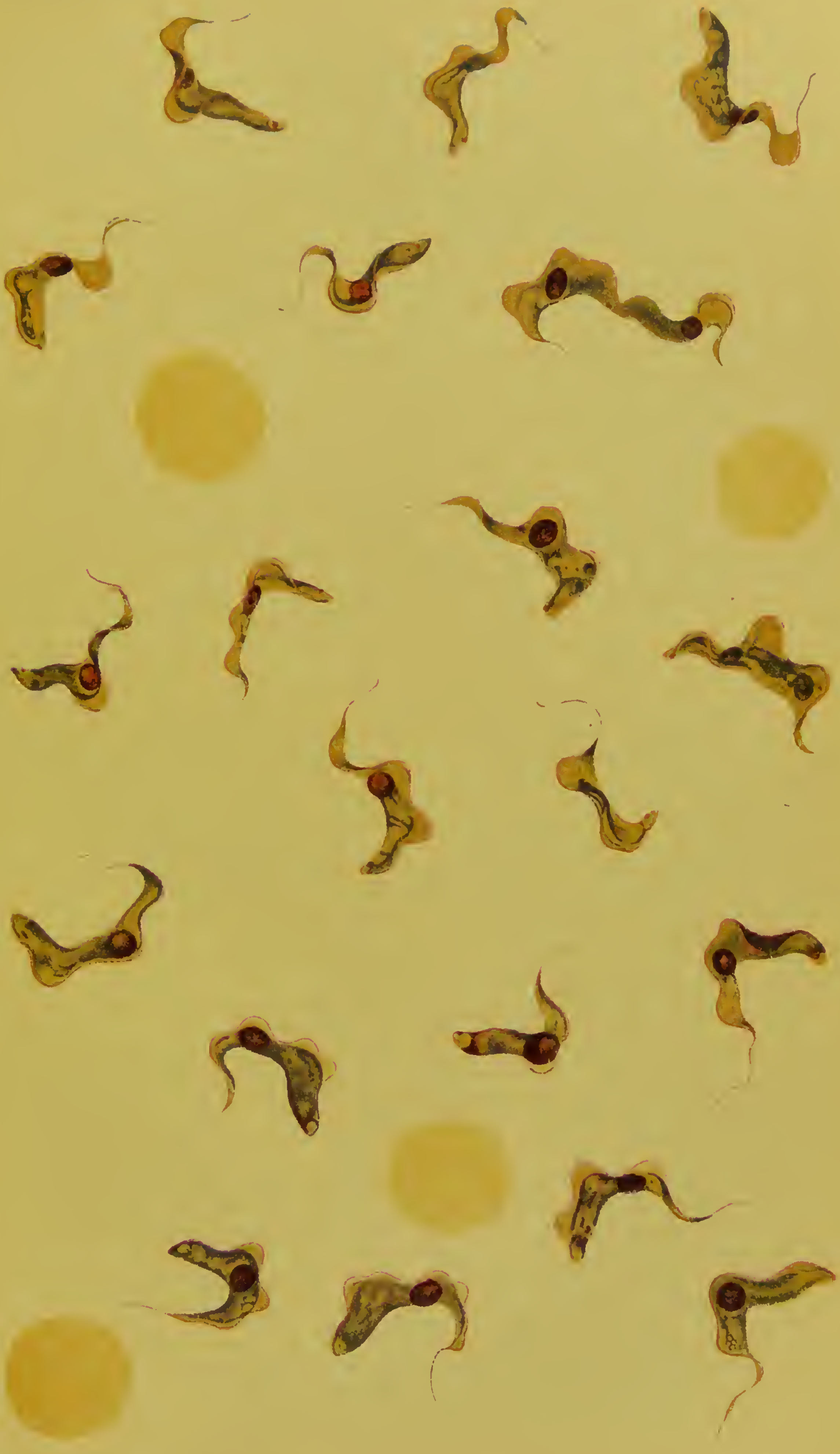
By Colonel Sir DAVID BRUCE, C.B., F.R.S., Army Medical Service; Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, Royal Army Medical Corps; and Captain F. P. MACKIE, Indian Medical Service. (Sleeping Sickness Commission of the Royal Society, 1908-09.)

(Received November 16,—Read November 25, 1909.)

During the last two years the policy of clearing the shores and islands of Lake Victoria of their inhabitants has been carried out by the Uganda Administration, with a view to the stamping out of Sleeping Sickness.

It will be remembered that the area of distribution of Sleeping Sickness and of the *Glossina palpalis* in Uganda is the same, and is limited to a narrow belt along the Lake-shore and islands. For the past two years no native has been allowed to live or work within two miles of the Lake-shore, except at a few cleared landing-places; and within the last few months all the islands have been emptied.

Until recently it was believed that the fly only retained its infectivity for 48 hours, and that it would, theoretically, be possible with safety to clear an island of its infected population one day and restock it with healthy natives a few days later. Recent work, however, has shown this to be wrong, since it has



Serg^t Gibbons, R.A.M.C. del.

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been found by experiment that the fly can retain its infectivity up to 80 days. It is probable that after a fly has become infected it will harbour the trypanosomes for the rest of its life; but what the duration of this is, under natural conditions, is unknown.

From an administrative point of view, therefore, it is most important to find out how long the flies on the Lake-shore remain infective after the native population has been removed. Until this is known it will not be safe to allow the Lake-shore and islands to be re-inhabited.

As soon as the Sleeping Sickness Commission of the Royal Society reached Uganda experiments were begun to test this point. At first the flies were collected at Kibanga, a cleared landing-place in Buka Bay, six miles from the laboratory. This landing-place was used as a market, where the inhabitants of the Island of Buvuma came once a week to trade with the natives on the mainland. In November, 1908, Kibanga had become somewhat overgrown, and tsetse flies were present in some numbers. As the Buvuma islanders were highly infected with Sleeping Sickness, this constituted a danger to the healthy natives of the mainland, who had come to the market from outside the Sleeping Sickness area. Steps were at once taken to have the landing thoroughly cleared of undergrowth, with the result that in a short time the flies disappeared.

The following experiment shows the result:—

Experiment 52.—Monkey.

To ascertain if *Glossina palpalis* caught at Kibanga market-place are capable of giving rise to Sleeping Sickness in a healthy monkey.

Date.	No. of Flies.		Trypanosomes.	Malaria.	Date.	No. of Flies.		Trypanosomes.	Malaria.
	Put on.	Fed.				Put on.	Fed.		
1908.					1908.				
Nov. 3	—	—	—	+	Dec. 6	—	—	—	+
" 6	—	—	—	+	" 7	—	—	—	+
" 14	15	12	—	+	" 15	—	—	—	+
" 16	17	17	—	+	" 17	1	1		
" 17	7	7	—	+	" 18	—	—	—	+
" 18	4	1	—	+	" 23	—	—	—	+
" 19	7	4	—	+	" 26	—	—	—	+
" 20	—	—	—	+	" 30	—	—	—	+
" 22	50	34	—	+					
" 23	—	—	—	+	1909.				
" 24	—	—	—	+	Jan. 4	—	—	—	+
" 25	—	—	—	+	" 9	—	—	—	+
" 27	—	—	—	+	" 18	—	—	—	+
" 29	—	—	—	+	" 20	—	—	—	+
" 30	—	—	—	+	" 26	—	—	—	+
Dec. 2	10	7	—	+	" 28	—	—	—	+
" 3	12	5	—	+	Feb 6	—	—	—	+
" 4	5	3	—	+	Mar. 1	—	—	—	+

Remarks.—The result of this experiment is negative. The number of flies caught is small, and they soon disappeared as the clearing of the place proceeded.

The other experiments were all made with freshly-caught flies from uninhabited places on the Lake-shore. The Lake-shore, as stated above, had been cleared of its inhabitants in December, 1907, and had, therefore, been deserted for nearly a year when these experiments began. It was anticipated that the flies would be found non-infective, as, in the absence of Sleeping Sickness cases, it was difficult to understand where they could obtain the necessary trypanosomes, and at this time the long period of infectivity of the fly was unknown. The following experiments give the result:—

Experiment 214.—Monkey.

To ascertain if *Glossina palpalis*, caught on the Lake-shore, where there are no natives, are capable of giving rise to Sleeping Sickness in healthy monkeys.

Date.	No. of Flies.		Trypanosomes.	Malaria.
	Put on.	Fed.		
1908.				
Nov. 23	21	10		
,, 24	25	20		
,, 25	50	26	—	+
,, 26	30	17	—	+
,, 27	12	8		
,, 28	96	23	—	+
,, 30	125	41	—	+
Dec. 1	150	60	—	+
,, 2	—	—	—	+
,, 3	—	—	—	+
,, 4	—	—	—	+
,, 5	60	23	—	+
,, 7	47	26	—	+
,, 12	60	49	—	+
,, 14	83	37		
,, 15	78	32	—	+
,, 17	14	6		
,, 18	—	—	—	+
,, 23	—	—	—	+
,, 28	80	35	—	+
,, 30	70	32	—	+
1909.				
Jan. 4	—	—	—	+
,, 9	—	—	—	+
,, 15	—	—	—	—
,, 25	43	24		
,, 26	35	29	—	+
,, 28	—	—	—	+
Feb. 2	100	65	—	+
,, 3	105	105		
,, 4	100	90		
,, 5	—	—	—	+
,, 6	100	85		
,, 8	100	82		
,, 9	200	165		
,, 10	200	146	—	+
,, 15	200	135	—	+
,, 16	200	120		
,, 17	170	126		
,, 18	200	134		
,, 19	200	110	—	+
,, 20	200	124		
,, 22	130	98		
,, 23	200	140		
,, 24	200	142		
,, 25	200	135		
,, 26	—	—	—	+
Mar. 1	—	—	+	+

Remarks.—2,500 flies were fed on this monkey for 98 days before a positive result was obtained.

Experiment 571.—Monkey.

Date.	No. of Flies.		Trypanosomes.	Malaria.	Date.	No. of Flies.		Trypanosomes.	Malaria.
	Put on.	Fed.				Put on.	Fed.		
1909.					1909.				
Mar. 2	200	152			Mar. 15	200	152		
,, 3	200	156			,, 16	100	78		
,, 4	100	78			,, 17	100	74		
,, 6	150	110			,, 18	100	58	—	+
,, 9	200	120	—	—	,, 20	200	112		
,, 10	200	110			,, 22	—	—	+	+
,, 11	200	124							

Remarks.—Result positive. Infection probably took place on March 15. This means that 1,002 flies fed on this monkey before infection took place.

Experiment 612.—Monkey.

Date.	No. of Flies.		Trypanosomes.	Malaria.	Date.	No. of Flies.		Trypanosomes.	Malaria.
	Put on.	Fed.				Put on.	Fed.		
1909.					1909.				
Mar. 25	340	185			Mar. 29	100	76		
,, 26	250	124			,, 30	200	115	—	+
,, 27	200	115			April 6	—	—	+	+

Remarks.—Result positive. Infection probably March 30; 615 flies.

Experiment 674.—Monkey.

Date.	No. of Flies.		Trypanosomes.	Malaria.	Date.	No. of Flies.		Trypanosomes.	Malaria.
	Put on.	Fed.				Put on.	Fed.		
1909.					1909.				
April 8	250	160			April 23	270	180		
,, 9	500	240			,, 26	200	160	—	—
,, 10	500	220			,, 28	400	240		
,, 12	500	245	—	+	,, 30	400	160		
,, 15	500	340			May 1	500	290		
,, 19	—	—	—	—	,, 3	—	—	—	+
,, 20	250	180			,, 7	—	—	++	+
,, 22	300	190	—	—					

Remarks.—Result positive. Infection April 30; 2,315 flies.

Experiment 758.—Monkey.

Date.	No. of Flies.		Trypanosomes.	Malaria.	Date.	No. of Flies.		Trypanosomes.	Malaria.
	Put on.	Fed.				Put on.	Fed.		
1909.					1909.				
May 8	270	210			May 22	—	—	—	+
,, 11	250	170			,, 28	200	130	—	+
,, 14	200	120			June 2	—	—	—	+
,, 17	—	—	—	+	,, 7	—	—	+	+

Remarks.—Result positive. Infection May 28; 630 flies.

Experiment 976.—Monkey.

Date.	No. of Flies.		Trypanosomes.	Malaria.	Date.	No. of Flies.		Trypanosomes.	Malaria.
	Put on.	Fed.				Put on.	Fed.		
1909.					1909.				
June 9	800	260			June 18	200	90	—	+
,, 10	450	180			,, 20	520	230		
,, 17	550	190			,, 21	—	—	++	+

Remarks.—Result positive. Infection June 10; 440 flies.

Experiment 1117.—Monkey.

Date.	No. of Flies.		Trypanosomes.	Malaria.	Date.	No. of Flies.		Trypanosomes.	Malaria.
	Put on.	Fed.				Put on.	Fed.		
1909					1909.				
June 24	200	120			June 30	7	4		
,, 25	300	160			July 1	—	—	—	+
,, 26	150	80			,, 3	500	130		
,, 28	380	165			,, 5	500	220	+	+
,, 29	500	210							

Remarks.—Result positive. Infection June 28; 525 flies.

Experiment 1276.—Monkey.

Date.	No. of Flies.		Trypanosomes.	Malaria.	Date.	No. of Flies.		Trypanosomes.	Malaria.
	Put on.	Fed.				Put on.	Fed.		
1909.					1909.				
July 9	110	70			July 19	—	—	—	+
,, 12	500	230			,, 20	300	180		
,, 15	—	—	—	+	,, 22	—	—	+	+

Remarks.—Result positive. Infection July 12; 300 flies.

Experiment 1462.—Ox.

Date.	No. of Flies.		Trypanosomes.	Malaria.	Date.	No. of Flies.		Trypanosomes.	Malaria.
	Put on.	Fed.				Put on.	Fed.		
1909.					1909.				
Aug. 16	120	75	—		Aug. 20	170	80		
,, 17	410	250			,, 24	350	120	—	
,, 19	320	180	—		,, 26	—	—	+	

Remarks.—Result positive. Infection August 19; 505 flies.

Experiment 1465.—Ox.

Date.	No. of Flies.		Trypanosomes.	Malaria.	Date.	No. of Flies.		Trypanosomes.	Malaria.
	Put on.	Fed.				Put on.	Fed.		
1909.					1909.				
Aug. 27	150	90			Sept. 7	—	—	—	
,, 28	60	35			,, 9	30	19		
Sept. 4	230	170			,, 10	—	—	++	

Remarks.—Result positive. Infection September 4; 295 flies.

Experiment 982.—Ox.

Date.	No. of Flies.		Trypanosomes.	Malaria.	Date.	No. of Flies.		Trypanosomes.	Malaria.
	Put on.	Fed.				Put on.	Fed.		
1909.					1909.				
Sept. 11	45	36			Sept. 20	—	—	—	
,, 12	65	50			,, 21	115	85	—	
, 14	110	75			,, 22	180	145		
, 15	125	95			,, 23	410	380		
,, 16	420	160	—		,, 24	300	240	—	
,, 19	55	40			,, 27	370	230	++	

Remarks.—Result positive. Infection, September 19; 456 flies.

The following table summarises these results:—

Experiment.	Place.	No. of Flies. fed.	No. of Days before Infection took place.	Result.	Percentage of Infected Flies.*
52	Kibanga	91	—	—	—
214	Uninhabited Lake-shore.	2500	98	+	0·04
571	,, ,,	1002	20	+	0·10
612	,, ,,	615	12	+	0·16
674	,, ,,	2315	29	+	0·04
758	,, ,,	630	30	+	0 16
976	,, ,,	440	12	+	0·23
1117	,, ,,	525	11	+	0·19
1276	,, ,,	300	13	+	0·33
1462	,, ,,	505	10	+	0·19
1465	,, ,,	295	14	+	0·34
982	,, ,,	456	16	+	0·22

* This is calculated on the assumption that there is only one infected fly in each batch of flies used in an experiment.

It must therefore be concluded that the Glossina palpalis *on the uninhabited shores of Victoria Nyanza can retain their infectivity for a period of at least two years after the native population has been removed.* How much longer they will remain infective it is impossible to say, but it is obvious that these experiments should be continued, in order to answer this important question.

With the facts at our disposal it is not possible to account for this continued infectivity. It may be due to the duration of the life of these flies being more than two years—that flies which became infected before the natives left are still alive. Or, it is possible that the flies have lately fed on natives suffering from Sleeping Sickness, who have been passing in canoes from the islands to the mainland, or on natives who still frequent the

Lake-shore in spite of the prohibition. Thirdly, it might be explained, if any of our canoe-men or fly-boys had trypanosomes in their blood. Or, lastly, it is possible that the mammals and birds along the Lake-shore have become infected, and so act as a reservoir of the disease.

To these speculations it may be answered that it is not at all likely that these flies have the opportunity of becoming infected from passing canoes, which during the last two years have been few and far between, or to natives still frequenting the Lake-shore. Our canoe-men and fly-boys have been kept under careful supervision during the whole of the time, their blood constantly examined, and once a month blood from each of them injected into a healthy monkey. There remain, then, the two theories—long duration of life of the fly, and a local reservoir. The former cannot at present be answered, and there is no experimental proof of the latter, since the injection of the blood of the Lake-shore birds and mammals into susceptible animals has always, up to the present, given negative results.

[*Reprinted from the* PROCEEDINGS OF THE ROYAL SOCIETY, B. Vol. 82.]

36. GLOSSINA PALPALIS AS A CARRIER OF TRYPANOSOMA VIVAX IN UGANDA.

By Colonel Sir DAVID BRUCE, C.B., F.R.S., Army Medical Service; Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, Royal Army Medical Corps; and Captain F. P. MACKIE, Indian Medical Service. (Sleeping Sickness Commission of the Royal Society, 1908-09.)

(Received November 27,—Read December 9, 1909.)

One of the important trypanosome diseases of cattle in Uganda is that caused by *Trypanosoma vivax* (Ziemann). This species of trypanosome appears to be widely distributed in Central Africa. It has been reported from Senegal, the Sudan and Erythrea in the North, to Rhodesia in the South. It is fairly easily recognised on account of its extreme activity during life, its characteristic shape in stained specimens, and the fact that it only affects cattle, goats, and sheep; while monkeys, dogs, rabbits, guinea-pigs, rats, and mice are refractory. Its carriers have usually been reported as tabanus and stomoxys.

This short note is written to place on record that fact, that in Uganda the tsetse flies, *Glossina palpalis*, which are found in large numbers on the Lake-shore, are infected, not only by *Trypanosoma gambiense*, the cause of sleeping sickness, but also by *Trypanosoma vivax*. The first experiment which showed that these tsetse flies are infected with the latter trypanosome was the following:—

Experiment 1318.—Calf.

To ascertain if oxen will become infected by trypanosomes if allowed to feed in the "fly area."

July 12, 1909. A healthy calf was taken down to the Lake-shore at Kibanga and ferried across the bay to Nsonga, where tsetse flies are numerous. The flies were observed to feed on it in numbers. It was then brought back to Kibanga. In future this calf will be taken out every day by the fly-boys to different parts of the Lake-shore, where it will graze while the boys are catching tsetse flies.

August 8. Returned from Lake-shore to Mpumu.

August 11. *Trypanosoma vivax* present in the blood of this calf.

Remarks.—If the incubation period of this disease is assumed to be eight days, then this calf remained 19 days at the Lake-shore before it became infected. The proof that the trypanosome found in this calf's blood was *Trypanosoma vivax* and not *Trypanosoma gambiense* was the shape and appearance of the parasite, the fact that the calf's blood injected under the skin of two monkeys gave negative results, and, lastly, that 50 laboratory-bred flies fed on this calf afterwards infected a goat with *Trypanosoma vivax.*

Experiment 431.—Cow. (Mother of Calf, 1318.)

July 12, 1909. This cow accompanied her calf to Kibanga, and remained with it during the experiment.

August 8. Returned to Mpumu.

August 19. *Trypanosoma vivax* discovered in blood.

The following table shows the dates of examination:—

Date.	Parasites in Blood.	
	Piroplasma.	Trypanosoma.
1909.		
January 9	—	—
,, 20	—	—
,, 21	—	—
,, 28	—	—
February 2	—	—
,, 9	—	—
,, 26	—	—
August 9	—	—
,, 11	—	—
,, 19	—	+

Remarks.—It is possible that this cow became infected from her calf, but it is more probable that she became infected in the same way and about the same time as her calf.

The remaining experiments were carried out by bringing freshly-caught *Glossina palpalis* from the Lake-shore to the laboratory at Mpumu and placing them on healthy oxen.

Experiment 1462.—Bull.

To ascertain if freshly-caught *Glossina palpalis*, fed on healthy cattle, will give rise to any trypanosome disease.

Date.		Flies.		Result.	
		Put on.	Fed.	*T. gambiense.*	*T. vivax.*
1909.					
August	16	120	75	—	—
"	17	410	250		
"	19	320	180	—	—
"	20	170	80		
"	24	350	120	—	—
"	26			+	—
September	1			—	+
"	2			—	+
"	3			—	+
"	4			—	+
"	6			+	+
"	7			+	+
"	8			+	+
"	9			—	+

Remarks.—Both *Trypanosoma gambiense* and *Trypanosoma vivax* appeared in the blood of this bull.

Experiment 445.—Bull.

Date.		Flies.		Result. *Trypanosoma vivax.*
		Put on.	Fed.	
1909.				
September	28 ...	470	220	
"	29 ...	160	95	
"	30 ...	65	45	—
October	1 ...	300	180	
"	2 ...	400	280	
"	4 ...	500	300	—
"	5 ...	190	85	
"	7 ...	250	170	—
"	8 ...	300	165	
"	11 ...	100	70	—
"	15 ...	450	360	—
"	16 ...	470	350	
"	18 ...	—	—	+
"	19 ...	—	—	++

Remarks.—*Trypanosoma vivax* only appeared in the blood of this bull.

Experiment 1465.—Bull.

Date.	Flies.		Result.	
	Put on.	Fed.	*T. gambiense.*	*T. vivax.*
1909.				
August 27	150	90	—	—
,, 28	60	35	—	—
September 4	230	170	—	—
,, 7	—	—	—	—
,, 9	30	19		
,, 10	—	—	—	+
,, 11	—	—	+	—

Remarks.—Both *Trypanosoma gambiense* and *Trypanosoma vivax* appeared in the blood of this bull.

Experiment 982.—Bull.

Date.	Flies.		Result.	
	Put on.	Fed.	*T. gambiense.*	*T. vivax.*
1909.				
September 11	45	36		
,, 12	65	50		
,, 14	110	75		
,, 15	125	95		
,, 16	420	160	—	—
,, 19	55	40		
,, 20	—	—	—	—
,, 21	115	85	—	—
,, 22	180	145		
,, 23	410	380		
,, 24	300	240	—	—
,, 25	—	—	—	—
,, 27	370	230	+	—
October 14	—	—	—	+

Remarks.—Both *Trypanosoma gambiense* and *Trypanosoma vivax* appeared in the blood of this bull.

Conclusions.

1. The *Glossina palpalis* on the shores of Victoria Nyanza are infected, not only by *Trypanosoma gambiense*, but also by *Trypanosoma vivax*.

2. What the reservoir of the virus of *Trypanosoma vivax* is, is unknown, but the buffalo, waterbuck, and other antelope which live on the Lake-shore should be examined.

37. ON THE ACTION OF ANTIMONY COMPOUNDS IN TRYPANOSOMIASIS IN RATS: BEING A REPORT TO A SUB-COMMITTEE OF THE TROPICAL DISEASES COMMITTEE OF THE ROYAL SOCIETY.

By JOHN D. THOMSON, M.B., C.M., and ARTHUR R. CUSHNY, M.D., F.R.S.

(Received November 23, 1909,—Read January 20, 1910.)

The near chemical and pharmacological relation of arsenic and antimony suggested naturally the use of the latter in a disease in which the former has proved of value; but the irritant action of the ordinary antimony salts seemed to preclude their use by hypodermic injection. After repeated attempts to form organic compounds of antimony analogous to atoxyl, one of us (C.) submitted to Plimmer and Thomson, for experimental trial, a compound of glycine and antimony, and their results with it showed that antimony possesses trypanocidal properties. This antimony compound proved difficult to make and unstable, however, and these observers substituted for it tartar emetic; the results were satisfactory, and the following investigation was undertaken with the object of determining the limits of usefulness of other antimony compounds in these diseases. The experiments were made on rats infected with a strain of Nagana (*T. brucei*) obtained by the kindness of Sir J. McFadyean. This strain was fatal to rats within six days after inoculation, or within three days after trypanosomes appeared in the blood. The inoculation and treatment were carried out at the Lister Institute of Preventive Medicine.

In the use of the heavy metals it is generally recognised that the more readily dissociated compounds are liable to cause more local irritation than others, and where the local action is to be avoided, attempts are made to use compounds which are less immediately dissociated and pass into the general tissues in their original form, there to free the metallic ion compound which is necessary for the desired effect.

An example of this is offered in the substitution of atoxyl for the older arsenic preparations in the treatment of trypanosomiasis, for there is no question that the atoxyl owes its activity to the simpler compounds formed from it in the tissues. It is possible that, in addition to avoiding local irritation, such compounds as atoxyl may penetrate into cells which are not permeable by ordinary arsenic salts, and that the latter may in this way be liberated in cells to which they would not otherwise have had access.

With this in mind it seemed desirable to test the action of compounds in which antimony is more firmly combined than the ordinary salts, but very few organic combinations are available, and we have succeeded in obtaining only two such for investigation. Of these the first was tetraethylstibonium iodide ($(C_2H_5)_4SbI$), which was injected into seven Nagana rats in quantities up to 20 milligrammes, but had no effect whatever on the parasites, the injected animals dying at the usual time, and the blood being found to be swarming with trypanosomes. The other, diphenylstibinchloride ($(C_6H_5)_2SbCl_3, H_2O$), was kindly put at our disposal by Professor A. Michaelis, of Rostock, but proved quite devoid of action in quantities of 4 c.c. of a saturated solution in sodium carbonate, in which it is more soluble than in water. These compounds appear non-irritant, but the antimony is probably not freed in the tissues, the first compound in particular resembling the ammonium salts, which pass through the animal body without freeing nitrogen.

These compounds proving valueless for our purpose, it was determined to find in what form the antimony ion had to be liberated to be effective, and a number of compounds commercially obtainable were tested. Of these the potassium metantimoniate (SbO_2OK), injected in quantities up to 30 milligrammes, scarcely affected the trypanosomes in the blood; doses above 30 milligrammes were followed by the disappearance of the parasites from the circulation, but the rats became very ill and died within a few days, and *post mortem* enteritis and nephritis were found to have been developed. A preparation of antimony oxide (Sb_2O_3), stated to be in colloidal form, was obtained from Kalle and Co., but proved extremely irritating, and had a very low efficiency. The preparation contained 4·5 per cent. of antimony oxide only, and in quantities corresponding to 4 milligrammes of the oxide had little, if any, effect in reducing the number of trypanosomes in the blood; 8 milligrammes caused their disappearance, but they recurred on the fourth day.

In the former of these preparations antimony is presented in pentavalent, in the latter in trivalent, form, and both are equally inefficient, while in the forms in which it is presented in combination with organic acids it is also in trivalent form, but has been shown to have a high degree of activity. It is possible that, given in the colloid form, it is deposited locally and fails to reach the trypanosomes. This would also explain the intense local action, which was more marked from this than from any other preparation.

A glyceride of antimony, analogous to boroglyceride, was formed by heating glycerine with the oxide, and proved fairly effective in destroying the trypanosomes in the rats, but it was very irritant and caused hæmoglobinuria, and the solution in glycerine tended to deposit the oxide when diluted.

A few experiments were made with Schlippe's salt, sodium sulphantimonate ($(NaS)_3SbS$); it destroyed the trypanosomes in the rat very satisfactorily (*see* Table I.), but induced very considerable local reaction, and therefore appears to be precluded from use in therapeutics. This result agrees with that obtained by Broden and Rodhain* in man.

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* 'Arch. f. Schiffs- u. Tropenhygiene,' vol. 12, No. 14, 1908.

Table I.—Summary of Results of Treatment with Schlippe's Salt.

I.	II.	III.	IV.
Number of Rats treated.	Number of Rats that died without recurrence, but before any deduction could be made.	Number of Rats surviving over one month after cessation of treatment without recurrence.	Number of Rats in which recurrence occurred.
20	6	3	11

Of the rats included in Column III.: One survived 39 days, one 33 days, and one 76 days. The cause of death did not seem to be the disease or its treatment.

Of the rats included in Column IV.: Recurrence took place in 10, 21, 9, 10, 10, 13, 7, 13, 16, and 14 days, and all died within two weeks of the cessation of treatment from the recurrence (or from the last recurrence, for many had more than one recurrence), with the exception of one which was alive and well 244 days after cessation of treatment of the recurrence—the latter by another drug (sodium antimonyl tartrate).

In Schlippe's salt and in the glyceride, antimony is pentavalent, and these were efficient trypanocides, especially the former, while the metantimoniate, where antimony is also pentavalent, possessed a low efficiency.

The sulphantimoniate differs from the metantimoniate in its great instability, and it seems probable that the marked local reaction arising from it is due in great part to its being decomposed at the point of injection with the deposit of the insoluble antimony sulphide Sb_2S_3, which produces a slow, lasting reaction. Enough reaches the general tissues, however, to react with the parasites, and here its instability, permitting of its forming new compounds, renders it peculiarly active. The metantimoniate, on the other hand, is much more stable, and probably fails to be reduced to the trivalent form.

The greater number of our experiments were done with the combinations of antimony with the organic acids corresponding to the ordinary tartar emetic. Among these the best combinations were found to be those with oxyacids of the fatty series, those of the aromatic series proving much less soluble. Among the salts examined were the lactate, citrate, malate, and mucate, the results of which were compared with those obtained by the tartrate.

They were formed by boiling antimony oxide with the acids, and subsequently neutralising with sodium hydrate, or sometimes by forming the acid sodium salt, and boiling it with freshly prepared antimony oxide. The mucate, which was investigated with the idea that its multiple hydroxyl groups might prove to have special powers of retaining antimony in solution, may be dismissed, as, though powerfully trypanocidal, it induced very great local irritation. The citrate seemed to be inferior to the malates and tartrates, which were approximately equal in value.

Table II.—Summary of Results of Treatment with Sodium Antimonyl Malate.

I.	II.	III.	IV.
Number of Rats treated.	Number of Rats that died without recurrence, but before any deduction could be made.	Number of Rats surviving one month after cessation of treatment without recurrence.	Number of Rats in which there was recurrence.
23	9	5	9

Of the rats included in Column III.: One survived 241 days; one 113 days; one 115 days; one 66 days; and one was alive and well 235 days after cessation of treatment.

Of the rats included in Column IV.: Recurrence took place in 12, 11, 7, 17, 5, 11, 9, 36, and 10 days, and all died within two weeks of cessation of treatment of recurrence (or of the last recurrence), except one which lived 231 days after cessation of treatment of the recurrence by another drug (Schlippe's salt).

Among the tartrates and malates, the sodium and potassium salts were equally efficient trypanocides, and there was no appreciable difference in their local effects, but the substitution of an alkyl radical for the potassium or sodium of the salt seemed to be attended with some advantage. Solutions of ethylantimonyl tartrate were kindly prepared for us by Professor Collie by heating freshly precipitated antimony oxide with ethyl tartrate, to about 150° C. in sealed tubes. The solutions are distinctly acid, but can be neutralised or rendered slightly alkaline with ammonia, and can be sterilised by boiling without any cloudiness resulting. It is a very efficient trypanocide and causes no local irritation in the rat. After the injection of 1 c.c. of a 0·2-per-cent. solution* into rats of 100 to 200 grammes weight, trypanosomes, though previously numerous, entirely disappear from the peripheral blood within one to two hours. Estimated by the amount of antimony present in the solutions, the ethyl is more powerfully trypanocidal than the sodium salt, which may suggest either that less is deposited at the point of application, or that it reaches the trypanosomes in a more readily penetrating form.

Though more poisonous to the rat than sodium antimonyl tartrate, the *range* of dose, or the difference between the effective trypanocidal dose and the lethal dose, is not less than that of the other antimony preparations. The optimum dose may be found to correspond to Browning's therapeutic dose, viz.: two-thirds of the maximum dose that average animals tolerate.

From our own work, and that of others, we think that sufficient data have been obtained to indicate some points which must be taken into consideration in attempting further advance in the treatment of trypanosomiasis:—

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* The strength of the solution was ascertained by estimation of the antimony

(1) As regards the compound it must be non-irritant and capable of remaining in perfect solution at the temperature and alkalinity of the tissues.

(2) It must act quickly on the trypanosomes, for otherwise these parasites seem to acquire a tolerance to it. It is possible that some drugs may require to be altered in the tissues before they begin to affect the parasites; but with this proviso, we suggest, as a working rule, that no drug which, given in full therapeutic dose, does not destroy the trypanosomes in the blood within two hours is likely to prove an advance on remedies already in use.

(3) When the trypanosomes have been expelled from the blood by a single full therapeutic dose, there must be no recurrence in the majority of cases within some fixed time, which will vary with the particular host, and the species and strain of trypanosome in use; the length of this period must be determined by each investigator by reference to some of the known trypanocides. In our experiments it proved waste of time to persevere with any drug whose administration in a single full dose was followed by a recurrence in the majority of cases within a week. The longer the time during which there is no recurrence in the majority of animals treated the more promising is the outlook for the drug under trial. But the non-recurrence in a single individual is of comparatively small importance.

In our experiments the majority of the rats that survived the injection for three weeks showed recurrence within that time, except when ethyl antimonyl tartrate was employed, when the results were slightly more favourable.

In the recent report* from Uganda, the conclusion is drawn that the medicinal treatment pursued up to that time had proved of little lasting benefit in the great majority of even the early cases of sleeping sickness. From this it seems a fair inference that remedies which suffice to change an acute trypanosomiasis into a more chronic form may ameliorate symptoms, but do not necessarily greatly prolong the natural course of a chronic infection such as sleeping sickness. In seeking for a remedy for the chronic condition, by experiments with acute infections, nothing short of an immediate and complete disinfection should be the object, and it is with this view that we venture to suggest the foregoing considerations.

Applying these principles to the ethyl antimony compound, it appears to comply with the first fairly satisfactorily. It expelled the trypanosomes from the blood within two hours. As regards recurrence after a single full dose, of 13 rats inoculated, 6 showed a recurrence, on the 14th, 16th, 16th, 22nd, 26th, and 29th day. There was no recurrence in the 7 others.

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* Quarterly Report on the Progress of Segregation Camps and Medical Treatment of Sleeping Sickness in Uganda (Quarter December 1, 1907, to February 29, 1908).

Table III.—Summary of Results of Treatment with Ethylantimonyl Tartrate.

I.	II.	III.	IV.
Number of Rats treated.	Number of Rats that died without recurrence, but before any deduction could be made.	Number of Rats surviving over a month after treatment without recurrence.	Number of Rats in which there was recurrence.
13	0	7	6

Of the rats included in Column III., one died of pneumonia on the 84th day, and five of the others from exposure to cold between the 135th and 165th day, leaving one survivor after 260 days.

Of the rats in Column IV., recurrence took place after 14, 16, 16, 22, 26, and 29 days respectively.

No other drug has given such favourable results in our experiments, but we recognise that it will be necessary to test it against other species and strains and in different hosts before any general statement as to its usefulness can be made.

We have also treated some rats with a solution combining the best of the arsenical and antimonial trypanocides at present known to us, viz., ethylantimonyl tartrate and atoxyl. Twelve rats were inoculated with the Nagana strain, and on the third day after, when a fair number of trypanosomes were present in the blood, they received a single injection of 0·75 c.c. of a solution containing 2 per cent. of atoxyl dissolved in 0·2 per cent. of ethylantimonyl tartrate.

Table IV.—Summary of Results of Treatment with Atoxyl + Ethylantimonyl Tartrate.

I.	II.	III.	IV.
Number of Rats treated.	Number of Rats that died without recurrence, but before any deduction could be made.	Number of Rats surviving over a month after treatment without recurrence.	Number of cases of recurrence.
12	2	8	2

Of the rats included under Column III., two died in 31, one each in 39, 43, 56, 89 days, and two survived after 260 days. Thus, while the number of recurrences was fewer than from the antimony alone, the combination of the two drugs seemed to tend to be toxic. This was more obvious in a series of 19 rats treated with a single injection of 0·75 c.c. of 3-per-cent. atoxyl in 0·3-per-cent. ethylantimonyl tartrate solution. Of these, 11 died within 24 hours of the injection, and as the blood was free from trypanosomes, they evidently succumbed to the treatment.

Table V.—Summary of Results of Treatment with Larger Doses of Atoxyl and Antimony.

I.	II.	III.	IV.
Number of Rats treated.	Number of Rats that died without recurrence, but too soon for any deduction.	Number surviving over a month without recurrence.	Number of cases of recurrence.
19	16	2	1

Of the two rats included in Column III., one died on the 113th day, and one survived after 225 days. The recurrence noted in Column IV. was noted on the 25th day. One of those in Column II. had trypanosomes in the blood the day after treatment and died that day.

The results of the combined medication were thus scarcely superior to those of the ethylantimonyl tartrate alone, for though there were fewer récurrences when atoxyl was added, the mortality from poisoning was higher, so that only two survived for two months as against six of those treated with antimony alone. It is possible that by a more accurate adjustment of the two drugs the advantages of diminished recurrence and low toxicity might be combined.

38. REPORT ON A COLLECTION OF BLOOD-PARASITES MADE BY THE SLEEPING SICKNESS COMMISSION, 1908-09, IN UGANDA.

By E. A. Minchin, M.A., Professor of Protozoology in the University of London.

(Plates 7, 8, 9.)

The following report deals with a collection of slides sent home by Sir David Bruce from Uganda, and entrusted to me for further investigation by the Royal Society. The whole collection consisted of 27 slides bearing blood-smears taken from amphibia, reptiles, and birds (no mammals), all stained by the Romanowsky method, some with Giemsa's stain, others with Leishman's. I have no information as to the method by which the blood was fixed, but the films seem to have been prepared by the ordinary method of drying in air, followed by fixation with absolute alcohol or methyl-alcohol. In some cases the smears are covered, in others not.

With the slides were a number of coloured drawings by Lady Bruce of the parasites in the smears. Some of these drawings are reproduced here, together with a certain number drawn from the preparations by my assistant, Miss Rhodes. With regard to these drawings I wish to make one observation. All Lady

Bruce's drawings were stated to have been executed at a magnification of 2,000 diameters, a standard magnification which I always employ for blood-parasites of this kind, unless they are excessively minute. When, however, Miss Rhodes drew parasites from these slides at a magnification of 2,000, they came out appreciably larger than Lady Bruce's drawings of the same parasites. I then checked the magnification of Miss Rhodes's drawings carefully by the method of drawing the divisions of the scale of a stage-micrometer, using the same camera lucida, eye-pieces, objectives, and microscope, with the same length of tube, in short the same arrangements in every detail that Miss Rhodes had used in making her drawings; and I found that the magnification was as accurately 2,000 as it was possible to make it. In order to compare the magnifications of the two sets of drawings, Miss Rhodes drew with the camera lucida the outlines of some red blood-corpuscles of the small toad (*see* below), to compare with drawings of corpuscles from the same slide made by Lady Bruce. It was then found that the corpuscles drawn by Miss Rhodes at a magnification of 2,000 averaged 35 mm. in length, while those drawn by Lady Bruce averaged 29 mm. in length. From these measurements I calculate that Lady Bruce's drawings are really magnified between 1,600 and 1,700 diameters.

The simplest method of dealing with a collection of material of this kind is first to enumerate the hosts in systematic order, stating briefly the forms of parasites that occur in each, and then to deal with points of interest presented by the various forms of parasites. I may state at once that no new or unknown types of blood-parasites have been discovered in these slides, but nevertheless some of the observations are well worth recording; perhaps the most interesting result of this investigation is the comparison of Halteridia from a number of different species of birds, which has not, to my knowledge, been undertaken before. It will be seen that the Halteridia differ from one another in structural characters in a marked manner and to an extent which, I must confess, was a surprise to me.

I. The Hosts and their Parasites.

Amphibia.

This class of vertebrates is represented by one slide and a drawing, labelled "Small toad from Kibanga, 28.11.08." The slide is, however, a very interesting one, since the smear contains two forms of trypanosomes fairly abundantly. One of these trypanosomes (pl. 9, figs. 61-63) is very large and similar in every way to *Trypanosoma mega*, Dutton and Todd (1903, pp. 51-53, pl. II., fig. 4), which was discovered by these investigators in "small frogs caught in a marsh at McCarthy Island." The other trypanosome is much smaller (pl. 9, fig. 64) and different in its characters; it is also generally not so well preserved on this slide as the large form, and it is difficult to find good specimens of it to draw. In all the trypanosomes in this smear both the trophonucleus and the flagellum are faintly stained; the free flagellum can only be seen with critical illumination.

As regards the large type of trypanosome, its chief characters, in addition to its large size, are as follows: the trophonucleus is placed slightly behind the middle of the body, and appears in the Romanowsky-stained preparations as a large oval space, placed transversely and stretching across the whole width of the body, usually quite clear, but sometimes showing a few grains tinged a faint red (pl. 9, fig. 63). The surface of the pre-nuclear region of the body shows very distinct longitudinal striations in the form of dark streaks, stained bluish or purplish, alternating with light streaks, or perhaps more correctly spaces, which are broken up into a succession of alveoli by thinner transverse dark streaks connecting the thicker longitudinal streaks already mentioned. This system of striations is generally interpreted as a system of myonemes or contractile fibrillæ, the light interspaces being regarded as the contractile elements themselves; but the preparations I have before me suggest strongly that the dark streaks should be identified as the actual contractile elements, if anything. This question cannot, however, be decided by a stain so unreliable for finer details of structure as the Romanowsky combination, and no other preparations are available. The striations are continued over the nucleus into the hinder part of the body, where, however, they become less distinct and obscured by coarse granulations.

It is interesting to note that in the pre-nuclear region the striations sometimes run nearly parallel on the two surfaces of the body, and in other cases cross each other, those seen at the higher focus being often nearly at right angles to those at the lower focus, indicating clearly that in the latter case the body has a spiral twist more or less pronounced (pl. 9, fig. 62). These appearances were noticed also by Dutton and Todd.

The kinetonucleus is not very large, generally oval in outline, and stains deeply; it is always close behind the trophonucleus, and there is a long postnuclear region of the body, sometimes greatly drawn out and attenuated. The flagellum arises from close beside the kinetonucleus and runs along the edge of a fairly deep undulating membrane, finally ending as a free flagellum of moderate length. The undulating membrane shows about a dozen pleats, deeper posteriorly and shallower anteriorly. The course taken by the undulating membrane is very instructive when compared with the arrangement of the myoneme-striations (pl. 9, figs. 61, 62). In those specimens in which the striations are straight and parallel on the two sides of the body (fig. 61), the body lies in the form of a C, and the undulating membrane runs along the convex side of the curve. When, however, the myoneme-striations cross each other on the two surfaces of the body (pl. 9, fig. 62), the body itself has one or more S-like curves, and the undulating membrane, keeping always to the convex side of a curve, crosses over or under the body at each spot where the curvature changes. In all cases alike it can be seen that the undulating membrane is parallel to those myoneme-striations which are nearest to it at its line of attachment to the surface of the body, and that the differences in the course of the undulating membrane in different specimens are due simply to the degree of spiral twisting in the body indicated, as already stated, by the myoneme-striations; possibly, indeed, caused by the contractility of the myonemes themselves.

The other type of trypanosome on this slide, besides being much smaller (pl. 9, fig. 64), has a moderately-sized trophonucleus placed in the posterior half of the body and far removed from the kinetonucleus, which is relatively close to the posterior termination of the body, so that the post-nuclear portion of the body is short. The flagellum arises close to the kinetonucleus and the undulating membrane is not greatly pleated. The body does not show distinct myoneme-striations, but the cytoplasm presents the appearance of vacuoles which sometimes are arranged in longitudinal series, an arrangement perhaps due to the existence of myoneme-bands.

The question at once suggests itself, whether these two forms of trypanosome belong to the same or to different species. With regard to the large forms, trypanosomes of this type have been described by several observers from African frogs since they were first discovered by Dutton and Todd in 1903; for instance by Dutton, Todd, and Tobey (1907) and by Rodhain (1908), all of whom consider that both the *mega*-type and the other types of form observed in frogs are all simply developmental variations of the common *T. rotatorium,* and Laveran and Mesnil also incline to this view in their well-known work on trypanosomes (1907, pp. 465-473). It would hardly be profitable to discuss this question on the evidence presented by a single slide. I may remark merely that if all the various forms described from frogs belong to one species, then the two forms occurring on Sir David Bruce's slide may well be two forms of the same species also. On the other hand the frequent occurrence, to judge from the literature, of *Trypanosoma mega* from African frogs, and the fact that it has not been recorded, so far as I am aware, from European frogs, seems to me to indicate that *T. mega* is a species distinct at all events from the European *T. rotatorium.*

Reptilia.

(1) *Lacertilia.*—The collection contains one slide labelled simply "lizard" without any further indication as to the species; some drawings have been made from it by Lady Bruce (pl. 7, figs. 20-23), which show an infection with an intra-corpuscular pigmented hæmamœba of the type of the *Hæmamœba metschnikori* described by Simond from an Indian tortoise. Such forms are now known from various reptiles and have been placed by Willey in a separate genus *Hæmocystidium,* but the majority of writers include them in the comprehensive genus *Plasmodium* (*Hæmamœba*). In the present instance large free forms are also seen (pl. 7, fig. 20), which have evidently been liberated from the corpuscles, either by their own efforts, or it may be by rupture of the corpuscle in making the smear. The film is unfortunately a defective one; in many places the corpuscles are

run together, and only their nuclei are distinguishable; in other places there is much precipitate of stain. A great many of the corpuscles present the apearance of young *Hæmocystidia* in their interior, but I am inclined to think that these appearances are caused by deposits of the stain.

(2) *Ophidia.*—There are three slides of smears of snakes' blood, each with figures drawn from them. One of the slides is labelled "Puff Adder, 15.1.09," and the accompanying drawing shows a red blood-corpuscle and a peculiar cell, and is marked "evidently a white blood-corpuscle, not a parasite," an opinion with which I entirely agree. Another slide is labelled "small snake, 13.12.08," and the corresponding drawing is marked "strange cells seen in a small snake killed on Mpumu." Having compared the drawing with the specimen, the cells in question appear to me to be also large leucocytes filled with coarse red-staining grains obscuring more or less completely a faintly stained nucleus.

The third slide is labelled "snake-blood, 3.2.09," and the drawing shows typical hæmogregarines in blood-corpuscles and is marked "same parasite in liver with a few free forms." Miss Rhodes has drawn some more figures from the film. As will be seen from the drawings (pl. 7, figs. 1-4), the hæmogregarine is a broad, more or less bean-shaped form, occurring in various stages of growth. No vermicular forms were observed, either free or coiled up in the corpuscle. The younger forms are moderately stout and do not displace the nucleus of the corpuscle; on the other hand very broad forms occur which push the nucleus to one side; there is, however, no karyolysis. Some of the hæmogregarines are extremely vacuolated. They are also in many cases very full of coarse red-stained grains, which may or may not be chromatic in nature. The nuclei of the parasites vary greatly in size and appearance, as the drawings show.

(3) *Chelonia.*—There are two slides in the collection, with drawings made from them, the one labelled "Tortoise, 10.1.09," the other "Tortoise, 15.1.09." There is no indication as to the species of tortoise, nor even whether the two smears were from the same species; since, however, the two preparations show quite similar parasites, and it is reasonable to suppose that a difference in the species of the host would have been noted, it may be inferred that both smears are taken from the same species of tortoise.

Both the tortoise-smears show hæmogregarines, very abundantly, and trypanosomes, in scanty numbers and requiring some searching to find. The hæmogregarine presents itself under a variety of forms and phases, and is found both free and intra-corpuscular.

The intra-corpuscular forms of the hæmogregarine can be classified under three types; in all cases alike the nucleus of the corpuscle is displaced, but not karyolyzed. There are first of all a small number of young forms (pl. 7, fig. 5), remarkable for the size of the nucleus, which is both relatively and absolutely larger than that of the full-grown forms, appearing as a mass of chromatin-grains occupying nearly the whole body, and leaving only a small quantity of cytoplasm free at the two ends. Secondly, there are vermicular forms (pl. 7, figs. 6-8), curled up within the corpuscle in the characteristic manner; the nucleus in this form is generally placed near the point at which the body bends over, and is relatively small or of moderate size and usually compact; the cytoplasm stains a faint blue, as a rule, and may be quite free from granulations, but more usually contains numerous red-stained grains, sometimes aggregated in a dense clump at the thickest part of the body, where the entire cytoplasm may be stained red (pl. 7, fig. 7). The third form of parasite is broad and bean-shaped (pl. 7, figs. 10-13); the cytoplasm stains an intense blue and generally is free from red-staining grains, but the nucleus is very large, occupying the middle of the body, and appearing usually as a diffuse patch of chromatin-granules.

The free hæmogregarines (pl. 7, figs. 9, 14) found on these smears do not differ in type from the intracorpuscular forms, and they appear to me to have been set free passively, by the process of smearing out the blood on the slide, rather than by their own efforts. This conclusion is clearly indicated by the vermicular types; only in a single instance have I seen a vermicule stretched straight out, after the manner of such forms when truly free. All the other free vermicules that I have seen are curled up in just the same manner as when they are intra-corpuscular, and in many cases a delicate capsule can still be seen round them (pl. 7, fig. 9). I infer, therefore, that the free parasites in these slides have become so by rupture of the host-cell artificially.

The trypanosomes found on the slides do not call for any special remark; they are of fairly large size, and their general appearance is shown by fig. 65, pl. 9.

(4) *Crocodilia.*—The collection contains one slide, with a drawing labelled "crocodile, 3.1.09, liver." It shows a somewhat scanty hæmogregarine-infection (pl. 7, figs. 15-19). The intra-corpuscular forms are either bean-shaped or vermicular, and in the latter case they are curled up in the characteristic manner with a distinct capsule (fig. 16). The nucleus of the corpuscle is displaced, but not karyolyzed. The free forms are for the most part similar to those found in the corpuscles, but in one case I have found a straightened-out and apparently naturally-free vermicule (fig. 19).

BIRDS.

The bulk of the collection consists of smears of bird's blood, 19 smears in all, representing 10 species of birds. On these preparations all the familiar types of avian blood-parasites are represented, namely:—

(1) The type for which Labbé's name *Halteridium* (1894) is still the most distinctive, although the name which is at present believed to be most correct, according to the rules of zoological nomenclature, is *Hæmoproteus*. I shall refer to these parasites as Halteridia, without prejudice to the vexed question of their correct designation. Their distinctive features are as follows: they are intra-corpuscular parasites of the red cells, more or less

amœboid, and containing melanin-pigment; they do not displace the nucleus of the corpuscle but grow round it, assuming the characteristic halter-like form which is expressed by Labbé's name. When the blood is drawn and cooled down without being dried, the ripe gametocytes burst their corpuscles, and the male forms "flagellate," that is to say, throw off male gametes. This habit of flagellating, and the fact that they contain melanin-pigment, distinguishes Halteridia at once from Hæmogregarines.

(2) The form which Labbé named *Proteosoma*, of which the name certified to be correct is *Plasmodium* (or *Hæmamœba*) *præcox* according to some, *P.* (*H.*) *relictum* according to others. I shall refer to it simply as the Proteosoma-parasite. It agrees with Halteridium in all its characteristics except one by which it is easily distinguished; namely that the parasite displaces the nucleus of the host-cell, and appears as a compact mass occupying nearly the centre of the blood-corpuscle.

(3) The *Leucocytozoon* or parasite of the white corpuscles discovered by Danilewsky (by no means to be confused with the pseudo-leucocytozoa found in some mammals, a perfectly distinct type of parasite, hæmogregarine in nature). The true avian Leucocytozoon is a peculiar spindle-shaped body in its full-grown form, without melanin-pigment, and with the nucleus of the host-cell attached to one side of the body of the parasite and often greatly drawn out. The adult Leucocytozoa show well-marked male and female types, and when the blood is drawn, the full-grown forms burst their corpuscles and round themselves off, the male forms proceeding to "flagellate" just like the two parasites already mentioned. In blood-films the attached nucleus of the host-cell generally stains deeply and is very distinct, but the nucleus of the parasite itself stains feebly and is often difficult to make out. In the preparations upon which I am reporting the nucleus of the parasite is generally not visible, or appears as an indistinct, faintly-stained patch of pinkish colour.

(4) *Trypanosomes.*—The types of avian trypanosomes are dealt with by Laveran and Mesnil in their well known treatise (1907, pp. 439-456).

In addition to the above four types of Protozoan blood-parasites, filarial worms occur in several of the preparations in this collection. Since the practice of drying blood-smears and staining them by the Romanowsky method is a very defective technique for the study of the structure of filariæ, I shall content myself with merely noting their occurrence on these slides.

I proceed now to describe the preparations of birds' blood in order:—

(1) *Guinea-fowl* (*Numida ptilorhyncha?*).*—There are seven slides of the blood of the guinea-fowl, all of different dates. The first (15.11.08) shows a very scanty infection

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* The birds in this collection of slides are denoted only by popular names. Mr. R. Olgilvie Grant, of the British Museum of Natural History, has kindly furnished me with the scientific names. Those that are doubtful are marked ??, less doubtful ?.

of Halteridium. The second (27.11.08) shows Halteridium and Leucocytozoon, but the preparation is very defective and the blood in parts is hæmolyzed. The third (4.1.09) also shows a scanty infection of the same two parasites. The fourth (10.1.09) is a defective preparation; the drawing (pl. 9, fig. 72) from it represents a female Leucocytozoon which has rounded itself off, indicating that the blood was dried very slowly in making the film. The fifth (3.1.09) shows a fairly abundant infection of Halteridium, Proteosoma, and Leucocytozoon. The sixth (5.1.09) shows abundant Leucocytozoon. The seventh (15.12.08), finally, shows a very good infection with Proteosoma; there are often several parasites in a field of the immersion-lens. The parasites (pl. 8, figs. 27-32) are all in nearly the same stage, very young forms which have just entered the corpuscle and begun their growth. Sometimes also free groups of very small forms are seen, probably clusters of merozoites derived from recent multiplication by schizogony (fig. 32).

These preparations of the guinea-fowl contain, therefore, taken altogether, all the types of avian blood-parasites with the exception of trypanosomes. Wenyon (1908, p. 141) has, however, described trypanosomes from the guinea-fowl and has named the parasite *Trypanosoma numidæ*. The Leucocytozoon of the guinea-fowl has been named by Balfour *L. neavei*.

The occurrence of Halteridium in this bird has also been noted by Wenyon (l.c., p. 150), but the occurrence of Proteosoma has not, so far as I am aware been recorded; this parasite is, however, so common in birds that there is nothing remarkable in its occurrence in this instance. The Leucocytozoa mostly show the nucleus of the host-cell compact, not drawn out; as already stated, the nucleus of the parasite is not stained. In the Halteridia also the nucleus of the parasite is not to be seen in any of those which I have examined, and is not figured in Lady Bruce's drawings.

(2) *Bee-eater* (*Merops albicollis??*).—There are two slides labelled "bee-eater, 15.11.08." The smears contain trypanosomes, Halteridia, and filariæ.

The trypanosomes are of moderate size (pl. 9, figs. 66, 67), and rather broad forms with the posterior extremity greatly prolonged, so that it simulates a flagellum. The trophonucleus is not far from the kinetonucleus, and between the two is an appearance of a vacuole in some specimens (fig. 67). The flagellum is faintly stained and very difficult to make out even with critical illumination; I am inclined to think that it is longer than is shown in the drawing.

The Halteridia (pl. 8, fig. 33) show coarse pigment-grains, but no nucleus distinctly; I have only been able to make out in some of them red grains, possibly of the nature of chromatin, at the ends of the body.

(3) *Blue Plantain-eater* (*Corytheola cristata*).—There is one slide labelled "Blue Plantain-eater, 6.12.08." The smear contains Halteridia, trypanosomes, and filariæ.

The Halteridia (pl. 8, figs. 34-39) are abundant and of a distinct type, of even thickness, very slightly curved, with coarse pigment-grains, and with red-staining grains at the two ends of the body in addition to a more diffuse red patch which apparently represents the nucleus and is usually situated near the middle of the body.

The trypanosomes (pl. 9, fig. 68) are scanty and are of a stout fleshy type which has become considerably deformed, apparently, in the process of drying.

(4) *Red-crested Plantain-eater* (*Musophaga rossæ*).—There is one slide labelled "Red-crested Plantain-eater, Kibanga, 8.11.08." The smear contains abundant Halteridia, of quite a different type from those found in the last bird (pl. 8, figs. 49-52). The parasites are more or less crescent-shaped with pointed ends, often very much bent round the nucleus of the corpuscle. The pigment-grains are not very coarse, and there are no red-staining grains apart from the nucleus which appears as a faintly-stained red patch generally at the middle of the body, sometimes near to one end.

(5) *Francolin* (*Francolinus mulemæ??*).—One slide is labelled "Francolin, 15.12.08." The corresponding drawing is marked "trypanosomes," but no trypanosomes are drawn, only Halteridia, Leucocytozoa, and filariæ. The slide shows Halteridia and Leucocytozoa fairly abundantly, but I have not found any trypanosomes.

The Halteridia (pl. 8, figs. 53, 54) are peculiar forms, very vacuolated and with large clumps of coarse pigment-grains, but do not show any signs of chromatin in this preparation. The body stains a purplish colour and grows round the nucleus of the host-cell in such a way that its two opposite ends sometimes almost come into contact.

The Leucocytozoa are large forms showing well-marked male and female types (pl. 9, fig. 70). It is not possible, however, to make out their nucleus clearly.

(6) *Black and White Hornbill* (*Bycanistes subquadratus*).—A slide labelled "Black and white hornbill, 8.11.08," shows fairly numerous trypanosomes (pl. 9, fig. 69). They are of stout type, with the distinct kinetonucleus placed close to the pointed posterior end of the body; the trophonucleus and the flagellum are very faintly stained and difficult to make out clearly. They are probably deformed to some extent by the process of drying.

(7) *Collar-dove* (*Turtur semitorquata*). — A slide labelled "Collar-dove, 4.1.09," shows a scanty infection of Leucocytozoa (pl. 9, fig. 71). The parasite has the typical form and appearance. The nucleus of the host-cell is deeply stained and compact, but that of the parasite is indistinct; the cytoplasm of the parasite is very vacuolated.

(8) *Coucal* (*Centropus superciliosus?*).—There are two slides of the coucal, labelled respectively 8.11.08 and 27.11.08. Both slides show a distinct type of Halteridium fairly abundantly (pl. 8, figs. 55-60). The body of the parasite is stout and pale in colour. The pigment-grains are few in number but very coarse.

The nucleus is represented by a faint red patch varying greatly both in shape and position, being sometimes central, sometimes terminal. Double infections of the corpuscles are frequent, and the parasites are seen in various stages of growth.

(9) *Ibis* (*Ibis æthiopica?*).—A slide labelled "Ibis, 1.12.08," shows an abundant infection of most peculiar Halteridia (pl. 8, figs. 40-48). The bodies of the parasites are very irregular in form, often with peculiar processes resembling pseudopodia; occasionally, however, a compact sausage-like form (fig. 47) is found, perhaps representing a full-grown ripe gametocyte. The pigment-grains are fine and distributed for the most part in clumps at the surface of the body; the nucleus appears to be represented also by red-stained streaks and patches which are placed at the surface of the body. The parasites are in all stages of growth, and in one case I have found what I believe to be a young Halteridium in the act of invading a corpuscle (fig. 48); it is very difficult to be certain that this is not simply an artefact, a deposit or precipitate of the stain or the like, and I give the figure for what it is worth, but I believe that it really does represent a very young parasite.

(10) *Egyptian Goose* (*Chenalopex ægyptiaca*).—One slide labelled "Egyptian goose, 25.1.09," shows a fairly rich infection of Leucocytozoa. The preparation is somewhat defective and the blood is largely coagulated, having apparently dried too slowly; in consequence many of the Leucocytozoa have rounded themselves off and present appearances similar to that shown in fig. 72, pl. 9.

II. General Remarks.

The following is a list of the various types of blood-parasites represented in this collection, with their hosts:—

Hæmogregarines.

Snake (pl. 7, figs. 1-4).
Tortoise (pl. 7, figs. 5-14).
Crocodile (pl. 7, figs. 15-19).

Hæmocystidia.

Lizard (pl. 7, figs. 20-23).

Halteridia.

Guinea-fowl (pl. 8, figs. 24-26).
Bee-eater (pl. 8, fig. 33).
Blue Plaintain-eater (pl. 8, figs. 34-39).
Red-crested Plantain-eater (pl. 8, figs. 49-52).
Francolin (pl. 8, figs. 53, 54).
Coucal (pl. 8, figs. 55-60).
Ibis (pl. 8, figs. 40-48).

Proteosomata.

Guinea-fowl (pl. 8, figs. 25 and 27-32).

Leucocytozoa.

Guinea-fowl (pl. 9, fig. 72).
Francolin (pl. 9, fig. 70).
Collar-dove (pl. 9, fig. 71).
Egyptian Goose.

Trypanosomes.

Small Toad (pl. 9, figs. 61-64).
Tortoise (pl. 9, fig. 65).
Bee-eater (pl. 9, figs. 66, 67).
Blue Plantain-eater (pl. 9, fig. 68).
Francolin (?).
Black and White Hornbill (pl. 9, fig. 69).

Filariæ.

Bee-eater.
Blue Plantain-eater.
Francolin.

Of these various types of parasites, the Halteridia deserve special mention. Halteridia are known from a very large number of different species of birds, but not much has been done to distinguish the species of the parasites themselves, and they are generally all included under a single specific name *Hæmoproteus danilewskyi*. Only in a few instances have special names, such as *H. noctuæ*, *H. columbæ*, been given to them, and then rather on the unsafe ground of a difference in habitat, than from any difference in the parasites themselves. Wenyon (1908) remarks that Halteridium was met with in a number of birds on the White Nile, and points out that "the parasites differ according to the host, and probably do not all belong to one species." He figures a curious species from the Jabira Crane, and observes that this species differs from the type usually met with in birds, of which he figures two specimens (l.c., pl. XIII., figs. 21, 22), and states that it occurs in various birds, including the common sparrow of the Sudan and the Guinea-fowl; the form figured by Wenyon appears very similar to that occurring in the Guinea-fowl in this collection (pl. 8, fig. 24). I am not aware of any other attempt to distinguish species of Halteridium by morphological characters.[*]

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[*] Since this Report was sent to the press, I have received a memoir by Dr. J. Burton Cleland and Mr. T. Harvey Johnston, entitled "Descriptions of new Haemoprotozoa from Birds in New South Wales, etc." (Journ. and Proc. Roy. Soc. N.S.W. XLIII., 1909, pp. 75–96, with two plates and two diagrams), in which the authors describe and compare in detail the Halteridia of four species of Australian birds, namely, *Ptilotis chrysops*, *Philemon corniculatus*, *Geocichla lunulata*, and *Meliornis novae-hollandiae.* Characteristic differences are pointed out between the parasites in each species of bird, and the Halteridia are given in each case distinct specific "labels" derived from the generic name of the host, namely, *H. ptilotis*, *H. philemon*, *H. geocichlae*, and *H. meliornis*, respectively.

As a glance at plate 8 will show, the Halteridia of different species of birds show differences much too striking to be explained as due to differences of technique and preparation, on the one hand, or, on the other hand, to the modifying influence of a different host on the same species of parasite. The Halteridia differ, as can be seen, in form, size, and structural features such as characters of the cytoplasm, distribution of the melanin-pigment and of the chromatin, &c., making all due allowance for differences of age and sex in the Halteridia themselves. I think there can be no doubt that in the future it will become necessary to recognize distinct species of Halteridia characterized by definite structural peculiarities, and not by their habitat, since the same species may often occur, probably, as Wenyon supposes, in several species of birds.

A remarkable feature of these different Halteridia is the great variation seen in their nuclei. The nucleus of this type of parasite stains as a rule very feebly, and many of the preparations show none at all; in other cases the nucleus is visible as a pale pink or reddish patch, showing great differences in size, position, and appearance. Differences in size are probably sexual, when they occur in the same species; some of the appearances exhibited, however, are very peculiar, especially those presented by the Halteridia of the Ibis (pl. 8, figs. 40-48), where the nucleus appears to be represented by a small flattened red patch at the surface of the body. Unfortunately the Romanowsky stain is exceedingly misleading for nuclear structure, and it would not be safe to draw any conclusions from it. In the case of the Halteridium of the Little Owl (*Athene noctua*), preparations made by Woodcock and myself at Rovigno and stained by approved nuclear methods, such as Heidenhain's hæmatoxylin, show the nucleus simply as a round vesicle with a karyosome, just as in a trypanosome; few things are more remarkable or disconcerting than the discrepancy between the results given by the Romanowsky method and other stains.

There is, however, one point to which I may draw attention. A striking feature of the Halteridium of the Blue Plantain-eater (pl. 8, figs. 34-39) is the presence of numerous red-stained grains at the two ends of the body, in addition to a red patch which probably represents the nucleus. The grains occur very abundantly in a young individual, and I have some doubt as to whether they are of the nature of chromatin; the mere fact that they stain red by the Romanowsky method is no proof that they are truly chromatin. But the occurrence of these red-staining grains at the two ends of the body may perhaps explain a very puzzling fact in the literature of Halteridium. Labbé (1894) stated that Halteridium reproduced itself by a process of sporulation at the two ends of the body; he gave figures of Halteridia with small nuclei at the two ends of the body, and of a process of segmentation into merozoites in this region. No one has ever yet confirmed this observation or been able to see anything like it. It seems to me possible that what Labbé mistook for small nuclei at the ends of the body may have been red-staining grains such as those which are present in the parasite of the Blue Plantain-eater. I may mention that I have also seen similar appearances in Halteridia of the Blue Jay of the Sudan in a preparation made by Major Dansey Browning, R.A.M.C.

Lister Institute,
26th November, 1909.

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DESCRIPTION OF THE PLATES.

Figs. 1–8, 10, 11, 15–19, 27, 33–39, 41–43, 47, 48, 50–53, 56–65, and 68 are drawn by Miss Rhodes at a magnification of 2,000 linear. The remaining figures are drawn by Lady Bruce at a magnification of about 1,650 linear (*see* p. 74).

Plate 7.

Figs. 1–4.—Haemogregarine of a snake (p. 77).
Figs. 5–14.—Haemogregarines of a tortoise (p. 77).
Figs. 15–19.—Haemogregarines of a crocodile (p. 78).
Figs. 20–23.—Haemocystidia of a lizard (p. 76).

Plate 8.

Figs. 24–26.—From blood of "guinea-fowl, 3.1.09" (p. 79); 24, a halteridium; 25, a proteosoma (or a halteridium rounding itself off?): 26, a halteridium (or a proteosoma?) which has escaped from the corpuscle and rounded itself off.

Figs. 27–32.—From blood of "guinea-fowl, 15.12.08" (p. 80). Various stages of Proteosoma. 27, very young form; 28–30, older intra-corpuscular forms; 31, schizogony?; 32, free cluster of merozoites.

Fig. 33.—Halteridium of bee-eater (p. 80).
Figs. 34–39.—Halteridia of blue plantain-eater (p. 80).
Figs. 40–48.—Halteridia of ibis (p. 82).
Figs. 49–52.—Halteridia of red-crested plantain-eater (p. 81).
Figs. 53, 54.—Halteridia of francolin (p. 81).
Figs. 55–60.—Halteridia of coucal (p. 81).

Plate 9.

Figs. 61–64.—Trypanosomes of small toad (p. 74). 61, large form. The striations are only figured on one surface of the body, since it would have confused the drawing had those of both surfaces been drawn, owing to their being nearly parallel on the two surfaces. 62, large form. The striations are figured on both surfaces, in order to show the manner in which they cross each other, owing to the twisting of the body. 63, a portion of the body of a large form showing a small quantity of stain in the nucleus. 64, small form of trypanosome.

Fig. 65.—Trypanosome of tortoise (p. 78).

Figs. 66, 67.—Trypanosomes of bee-eater (p. 80).

Fig. 68.—Trypanosome of blue plantain-eater (p. 81)

Fig. 69.—Trypanosome of black and white hornbill (p. 81).

Fig. 70.—Leucocytozoon of francolin, male type (p. 81).

Fig. 71.—Leucocytozoon of collar-dove, female type (p. 81).

Fig. 72.—Leucocytozoon of "guinea-fowl, 10.1.09" (p. 80), female type which is in the act of rounding itself off.

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39. AMAKEBE: A DISEASE OF CALVES IN UGANDA.

By Colonel Sir David Bruce, C.B., F.R.S., Army Medical Service; Captains A. E. Hamerton, D.S.O., and H. R. Bateman, Royal Army Medical Corps; and Captain F. P. Mackie, Indian Medical Service. Sleeping Sickness Commission of the Royal Society, 1908—09.

(Received December 18, 1909,—Read January 20, 1910.)

[Plate 10.]

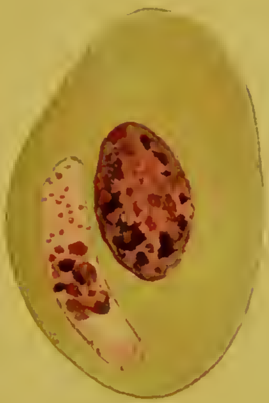
Introductory.

Amakebe is the most important disease of cattle in Uganda. It attacks the calves soon after they are born, and destroys more than half of them. Among the native cattle the loss is reported to be as much as 75 per cent., but, with careful nursing and hand-feeding, this mortality may be reduced to between 20 and 30 per cent. This is an enormous toll to pay, and renders the breeding of cattle in Uganda for dairy purposes, or, indeed, for any purpose, very up-hill work.

Little up to the present has been written as to the nature and causation of amakebe. It has been described as a trypanosome disease, but this evidently on insufficient knowledge.

Distribution in Uganda.

Amakebe appears to occur all over the Kingdoms of Uganda, Unyoro, Ankole, and Busoga. Lieutenant A. D. Fraser, Royal Army Medical Corps, the medical officer lately in charge of the Sleeping Sickness Camp, Sesse, reports, however, the curious fact that it does not occur among the cattle on the Sesse Islands. Mr. C. W. Hattersley also informs the Commission that cows brought to Mengo from Ankole invariably contract the disease, which would go to show that in some parts of Ankole the disease does not occur. Mr. R. J. Stordy, the chief veterinary officer, British East Africa, states that amakebe is found at every altitude in that Protectorate. Dr. A. Theiler, C.M.G., the chief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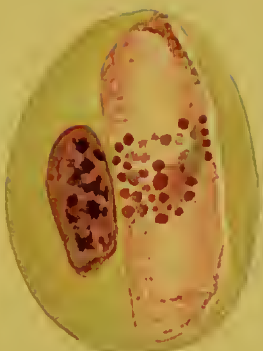
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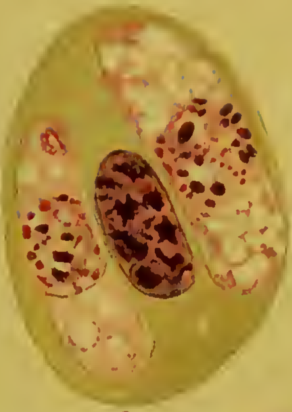
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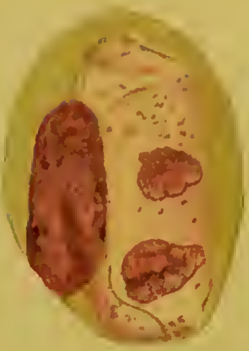
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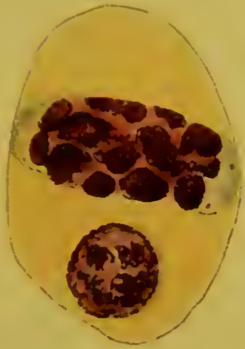
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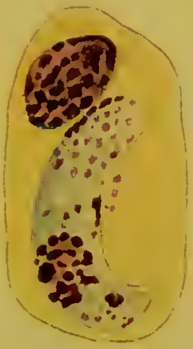
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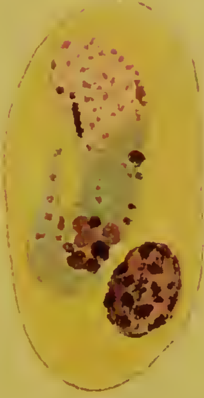
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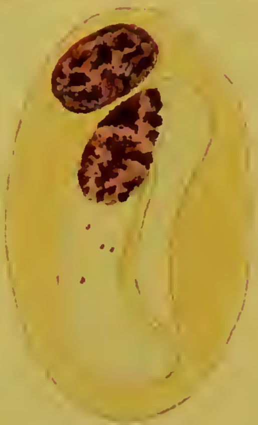
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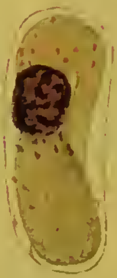
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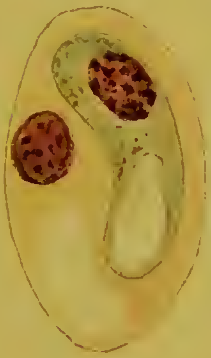
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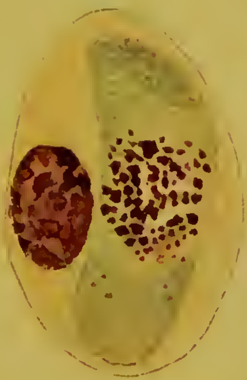
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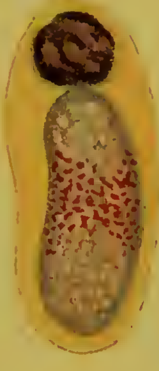
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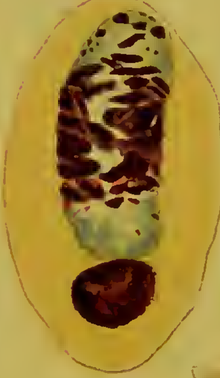
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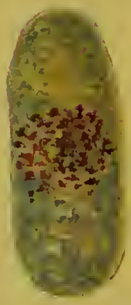
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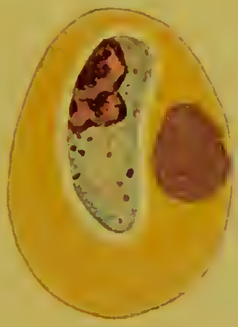
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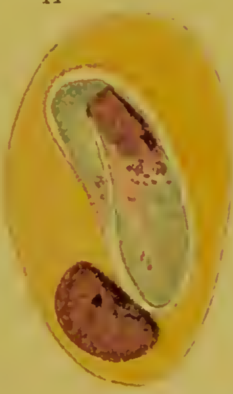
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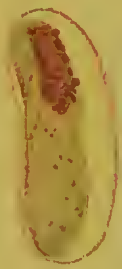
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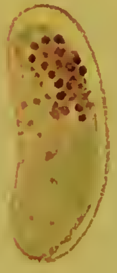
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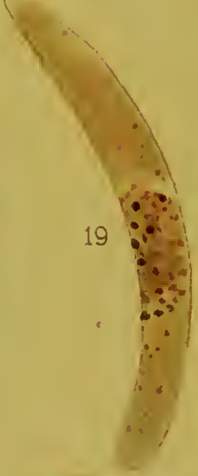
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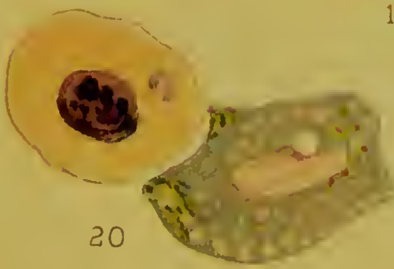
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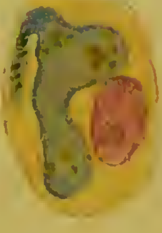
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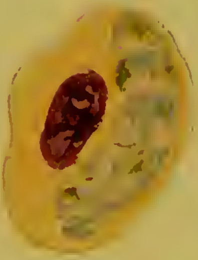
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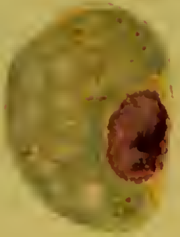
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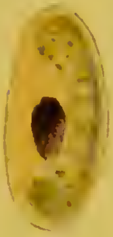
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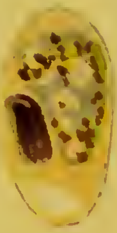
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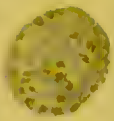
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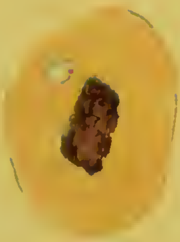
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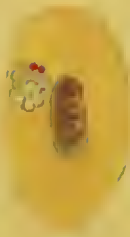
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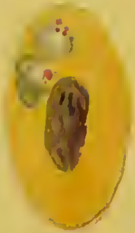
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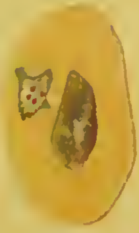
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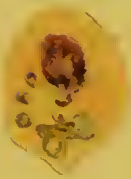
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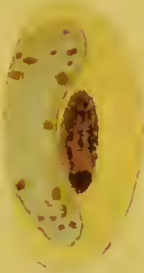
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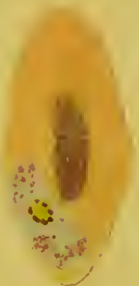
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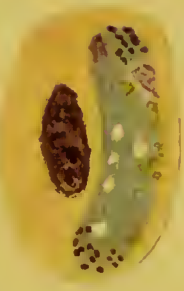
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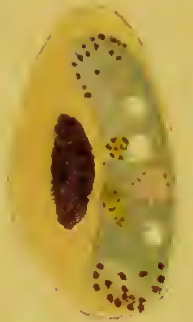
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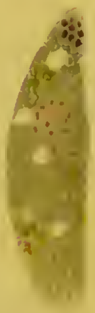
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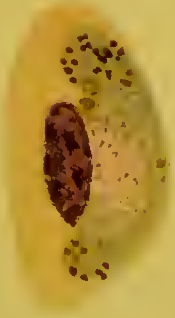
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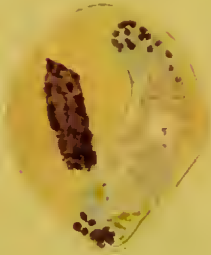
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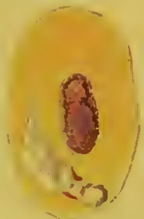
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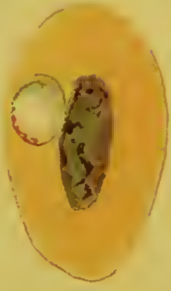
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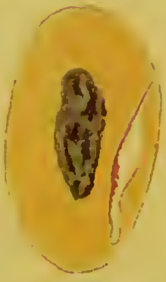
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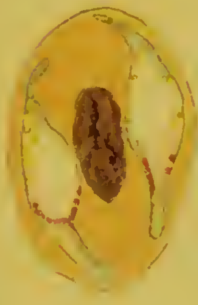
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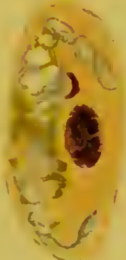
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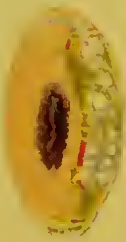
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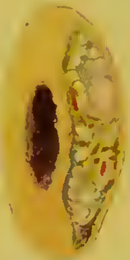
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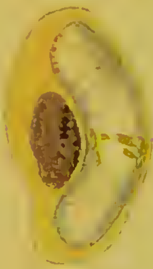
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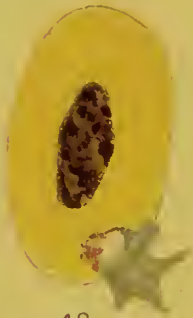
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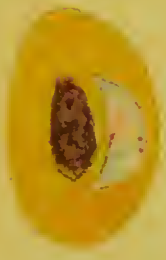
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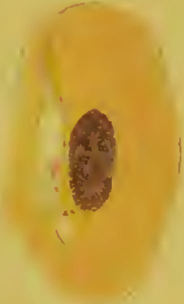
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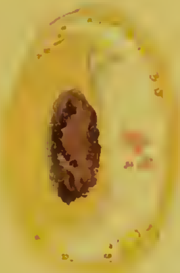
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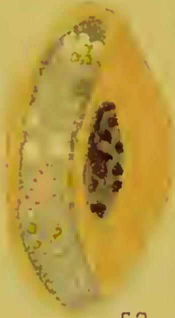
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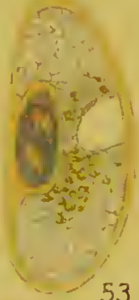
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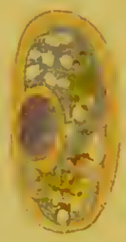
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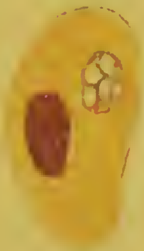
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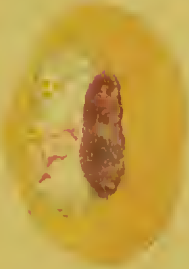
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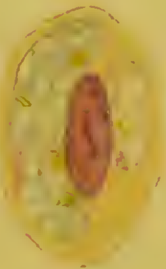
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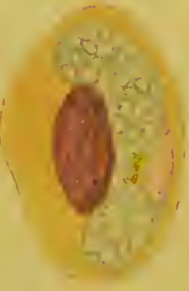
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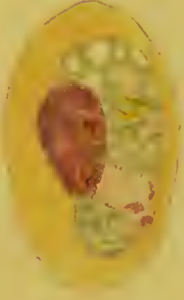
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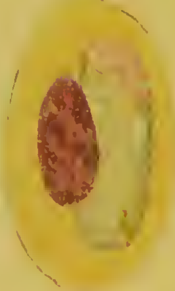
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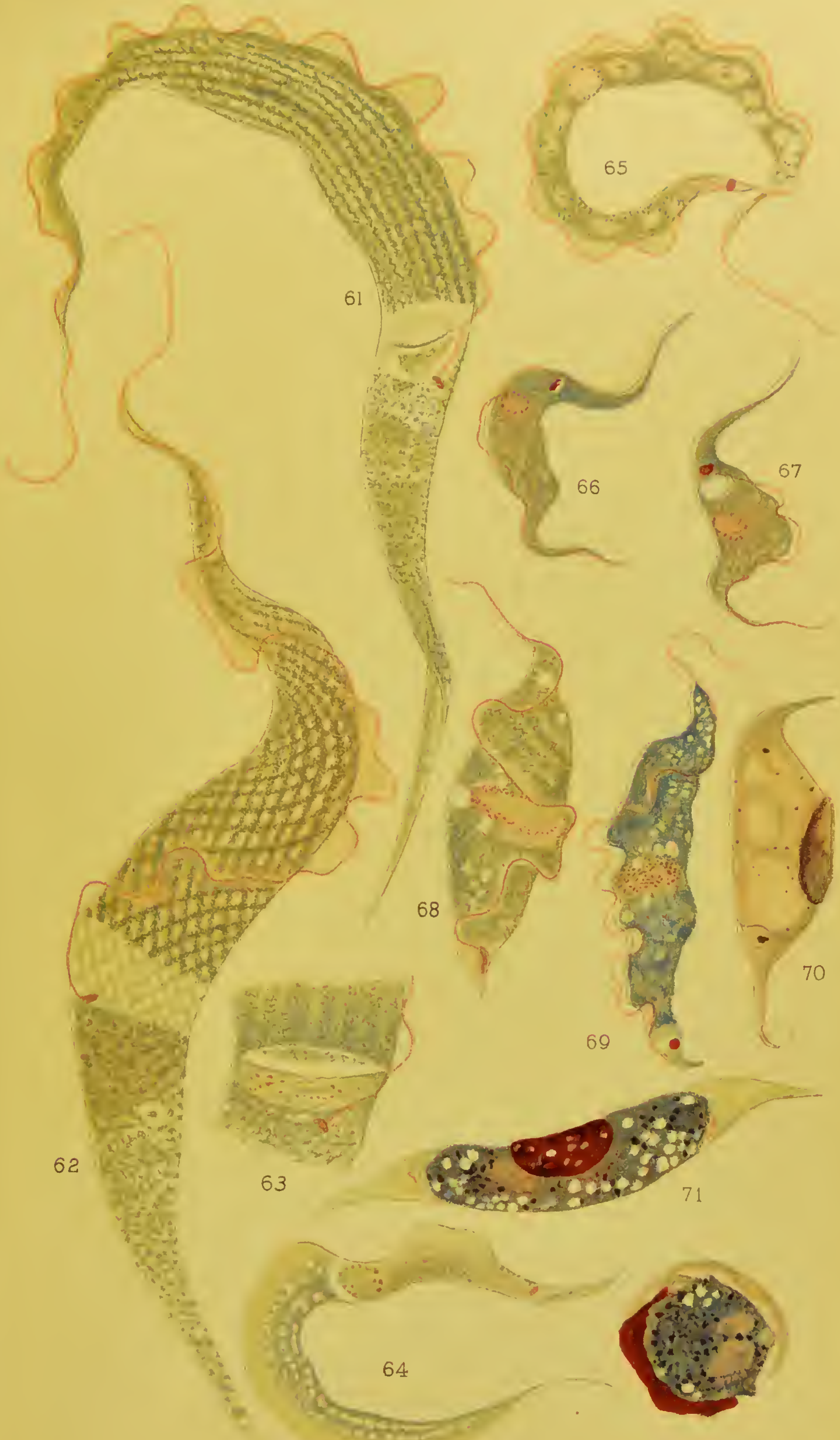
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veterinary bacteriologist, Transvaal, who lately visited Uganda, writes that Dr. Lichtenfeld, the principal veterinary officer, German East Africa, told him that a disease similar to amakebe exists in Ruanda, on the western shores of Victoria Nyanza and adjoining Ankole.

It is evident, then, that this disease is widely prevalent in Central Africa, and most disastrous in its effects.

Nomenclature.

In Uganda the disease is known as kebe, makebe, or amakebe, and means calves' swollen glands, or mumps. At Ngora, to the west of Mount Elgon, the natives call the disease angarwe. In Unyoro, masugu. In Ankole, amashuyu or amashui.

Symptoms.

The chief symptom of this disease is the swelling of the lymphatic glands, especially those in the region of the ear, in front of the shoulder, and in front of the hip. The glands frequently reach a large size, those in front of the shoulder often being three or four inches in length. They are soft to the touch, giving the impression of an elastic body under the skin. The hair is rough and staring, the head hangs, the ears droop, and there is frequently a watery discharge from the eyes and nose. During the illness the temperature runs high, often reaching 107° F. or more. The calf becomes rapidly emaciated, and often a dry scabby eruption of the skin is seen. Diarrhœa is frequent, and the dung is often dark in colour, with an evil odour. The urine never shows any trace of blood, as in redwater.

The duration of the disease is usually about a fortnight, but sometimes the calves get over it in three or four days. The fever goes, they pick up condition, and the swelling of the glands subsides. The glands, however, never regain their normal size, but remain permanently enlarged throughout life.

When a calf has recovered from amakebe it is no longer susceptible to the disease. It is immune for the rest of its life.

The following cases illustrate the course of the disease:—

Experiment 1,387.

To study Amakebe in the Calf.

July 26, 1909.—Animal received from Sir Apolo Kagwa, K.C.M.G., Kampala.

July 29.—The prescapular glands are the size of a walnut. The calf looks fairly well, is thin, and hair slightly rough.

Aug. 2.—The lymphatic glands are much more enlarged. The prescapular glands measure 3½ by 2 inches.

Aug. 12.—This calf is now looking very sick. Conjunctival mucous membrane congested. The hair is falling off in patches, leaving a rough, scabby surface. Diarrhœa.

Aug. 14.—Discharge from eyes and nose. Diarrhœa.

Aug. 26.—This calf got steadily worse, and died at 11.30 a.m. to-day.

The following chart represents the course of the temperature :—

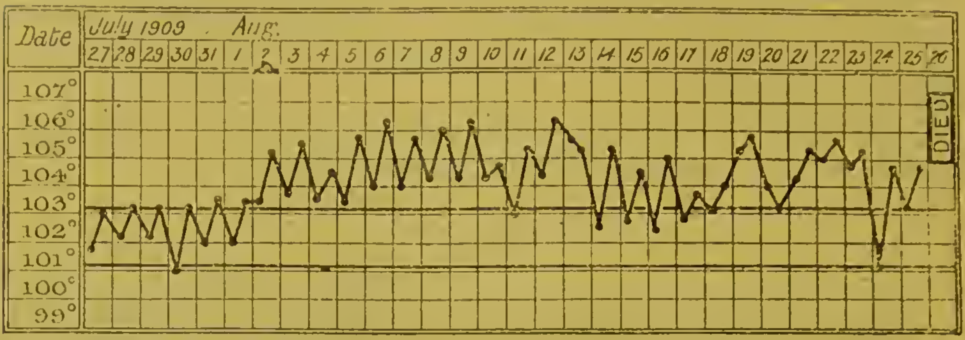


Chart 1.—Experiment 1,387. The Temperature Curve in a severe Case of Amakebe.

Aug. 26, 1909.—*Post-mortem* immediately after death.

External Appearances.—The body is emaciated. *Rigor mortis* absent. The hair is staring and has a ragged appearance. There are many patches of eruption on the surface of the body, especially on the face and head. These eruptions are, as a rule, about the size of half-a-crown and resemble limpet-shells.

Internal Appearances.—On removing the skin, the flesh is seen to be pale in colour. On opening into the abdomen the spleen is seen to be enlarged. The surface of the stomach and intestines is exceedingly pale and yellowish. There is no fluid in the peritoneal cavity. On opening into the thorax no fluid is found in either pleural cavity. There is about a tablespoonful of bright, chrome-coloured, clear fluid in the pericardium. The anterior mediastinum contains a quantity of bright yellow, jelly-like material. The serous membranes are shining and smooth.

Lymphatic Glands.—The lumbar chain of glands are enlarged, some of them being the size of a small walnut. These enlarged glands on being cut into are found to be very œdematous, but not hæmorrhagic. The prescapular glands are much enlarged, being 8 cm. (3½ inches) in length.

Circulatory System.—Heart.—The fat of the auriculo-ventricular groove is infiltrated with gelatinous material, which is bright yellow in colour. There are no petechiæ under the epicardium. On opening into the left ventricle many minute petechiæ are seen under the endocardium. The colour of the aorta is bright yellow. The substance of the heart is pale in colour and flabby to the touch. Weighs 125 grammes (4½ ounces).

Respiratory System.—Left lung is purplish in colour, with a dark purple patch in the anterior lobe about the size of half-a-crown. This, on being cut into, shows hæmorrhagic infarction. On section the left lung appears to be fairly healthy. Weighs 210 grammes (7½ ounces). *Right Lung.*—Anterior lobe and part of the middle lobe are purplish-red in colour and solid. The anterior lobe sinks in water. The surface is purplish-red in colour, and across the surface is a network of yellow-coloured, clear, jelly-like strands, resembling the lung in a case of horse-sickness. The strands in some places are ¼ inch wide. On section the substance is seen to be hepatised and dark purple in colour, and across the cut section the same network of yellow, gelatinous-like material is seen. The posterior lobe is pale in colour, and on section appears fairly normal. Weighs 345 grammes (12¼ ounces).

Alimentary System.—Spleen is enlarged. 29 cm. in length, 9 cm. broad, and 2·5 cm. in thickness (11″+3½″+1″). Capsule is purplish in colour. On section the tissue is dark purple in colour and friable. Weighs 245 grammes (8¾ ounces). *Liver* is bright yellow in colour, tinged with red, like bronze. Capsule is smooth. On section the substance is pale, with congested areas. *Gall-bladder* is distended with thick, greenish-yellow bile. Weighs 890 grammes (31½ ounces).

Fourth Stomach.—Is pale in colour. No ulceration. Intestines not examined.

Urinary System.—Left kidney.—Capsule strips readily. On section the cortical part is seen to be pale, with dilated vessels. Weighs 102 grammes (3½ ounces). *Right kidney*, in a similar condition to the left. Weighs 95 grammes (3¼ ounces).

Experiment 1,634.

To study Amakebe in the Calf.

Sept. 4, 1909.—This calf was brought to Mpumu from Kome, one of the Sesse Islands, and was therefore susceptible to amakebe.

Sept. 14.—Sent into Kampala, in order to become infected.

Sept. 24.—Returned from Kampala.

Oct. 4.—Lymphatic glands much enlarged.

Oct. 18.—Died.

The following chart represents the course of the disease:—

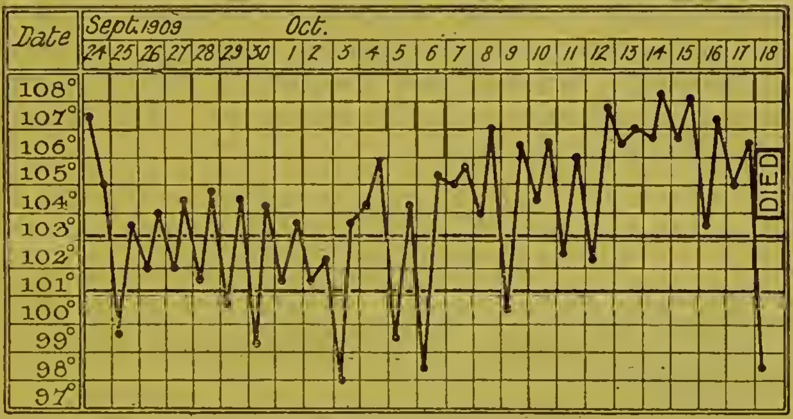


Chart 2.—Experiment 1,634. Temperature Curve in a severe and fatal Case of Amakebe.

Oct. 18, 1909.—*Post-mortem* immediately after death.

External Appearances.—Animal about one year old. Preauricular, prescapular, and precrural glands are much enlarged. The prescapular glands measure 3 inches in length and 1½ inches in breadth. On section the glandular tissue is œdematous and, in some places, hæmorrhagic.

Internal Appearances.—On opening into the peritoneal cavity about a gallon of clear, amber-coloured fluid is found. There is a large quantity of yellow, gelatinous infiltration into the omentum. The serous membrane of the omentum is markedly hæmorrhagic, being covered with small petechiæ. The small intestine is dark crimson in colour and intensely congested. The whole of the peritoneal aspect of the diaphragm is covered with small hæmorrhages. On removing the sternum a quantity of yellow, gelatinous material is found in the mediastinum. About 2 ounces of the same clear, amber-coloured fluid are seen in the pleural cavity. The pericardium contains a small quantity of clear, straw-coloured serum.

Circulatory System.—Heart.- A quantity of yellow, gelatinous material is seen at the base. Many small petechiæ both inside and outside the heart.

Respiratory System.—A quantity of white frothy fluid exuded from the nose during the last hours of life. On opening into the *trachea*, however, it is now found to be empty. The *left lung* is partially collapsed and is dark purple in colour. On section the organ is dark crimson in colour and intensely congested. It is, in places, solid in consistence and a portion placed in water sinks. On pressure a white, frothy fluid exudes. *Right lung* is pale in colour, and there are numerous hæmorrhages into the serous membrane. On section it is found to be congested. No part of the lung sinks in water.

Alimentary System.—Spleen is enlarged. Measures 14 inches in length and 4½ inches in breadth. Numerous petechiæ into the capsule. On section the substance is dark in colour, soft, and friable. Weighs 480 grammes (17 ounces). *Liver* is enlarged. On section is seen to be congested. Gall-bladder is distended with dark, olive-green-coloured bile. Weighs 3 lbs. 10 ozs.

Fourth Stomach.—Is reddened, and there are numerous small ulcers in the serous membrane.

Urinary System.—Right Kidney.—Capsule strips readily. There are numerous petechiæ into the capsule. Surface of the organ is injected. On section the kidney is seen to be congested, with many hæmorrhages into the substance. *Left kidney* is in a similar condition to the right.

Experiment 1,636.

To study Amakebe in the Calf.

Sept. 4, 1909.—From Kome. Same history as Experiment 1,634. Great enlargement of lymphatic glands.

Oct. 12.—Died.

The following chart represents the course of the disease:—

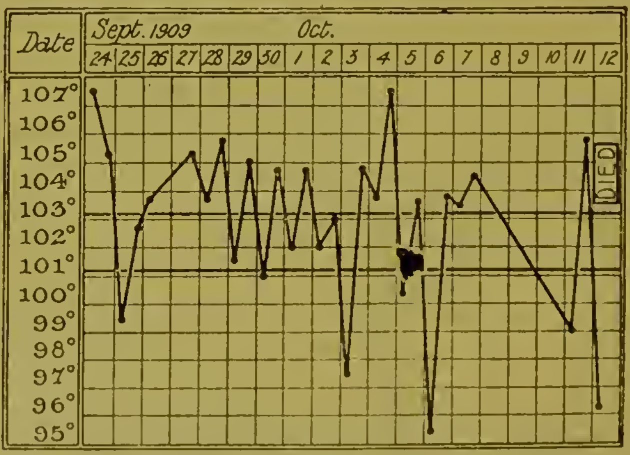


Chart 3.—Experiment 1,636. The Temperature Curve in a severe and fatal Case of Amakebe.

Oct. 12, 1909.—*Post-mortem* two hours after death.

External Appearances.—This calf has had a running from the nose of clear fluid, which has made a small pool under its head, and at death there was a marked collection of white foam at the nose, like that which occurs in horse-sickness, but not to such an extent.

Internal Appearances.—On removing the skin and opening into the peritoneum, about 4 ounces of clear, straw-coloured fluid is found. The omentum is infiltrated with a yellow jelly-like material. On opening into the thorax, 2 ounces of clear, straw-coloured fluid is seen in the pericardium. About 4 ounces of the same straw-coloured fluid in both pleural cavities. On removing the tongue and trachea a large quantity of jelly-like material is found under the trachea.

Lymphatic Glands. — The prescapular glands are enlarged. One measures 4½ inches in length and 1 inch in thickness. On section it is seen to be dark crimson in colour and hæmorrhagic. The glands generally are enlarged and hæmorrhagic. Some of them show signs of breaking down into pus.

Circulatory System.—*Heart.*—A large quantity of yellow, jelly-like material at the base is seen. There are numerous small petechiæ into the epicardium. None in the endocardium. Muscular substance is pale. Weighs 1 lb. 5 ozs.

Respiratory System.—*Tongue* normal. The *trachea* is full of white froth. Glands at the bifurcation of the trachea are much enlarged and hæmorrhagic. *Right lung*, anterior lobe is dark purple in colour and is found to be the seat of a large infarct. Posterior lobe is also the seat of an infarct at the margin, about 3 inches by 2 inches in extent. On section the substance of the lung is extremely œdematous. A large amount of frothy fluid exudes on pressure. Weighs 2 lbs. 10 ozs. *Left lung* is also the seat of numerous infarcts. On section a large amount of frothy fluid exudes on pressure. Weighs 1 lb. 6 ozs.

Alimentary System.—*Spleen* measures 13 inches in length and 4 inches in breadth. On section the substance is soft and friable Weighs 13 ounces. *Liver* is enlarged. It is full of flukes. The gall-bladder is distended with dark, chocolate-coloured bile, which contains many flukes. Weighs 8 lbs. 5 ozs.

Fourth Stomach.—The mucous membrane of the fourth stomach is congested. It is dark crimson in colour, and numerous small ulcers are seen scattered throughout.

Urinary System. — *Right Kidney.* — Capsule strips readily. Under the capsule numerous infarcts are seen, about the size of a pea. On section the substance of the organ is congested. Weighs 8 ounces. *Left Kidney.*—Capsule strips readily. In the pelvis of the organ there is a quantity of yellow, jelly-like material. Under the capsule there are also several small infarcts. One of these is as large as a small marble. On section the substance of the kidney is seen to be congested. Weighs 5 ozs.

Experiment 1,386.

To study Amakebe in the Calf.

July 26, 1909.—Received from Sir Apolo Kagwa. Had also been kraaled at Kampala for some days.

Aug. 27.—The course of the disease was much the same as in Experiment 1,387. The prescapular and other glands became much enlarged, one of them measuring 4 inches in length. By this date the calf had recovered, and was returned to owner.

The following chart represents the course of the temperature:—

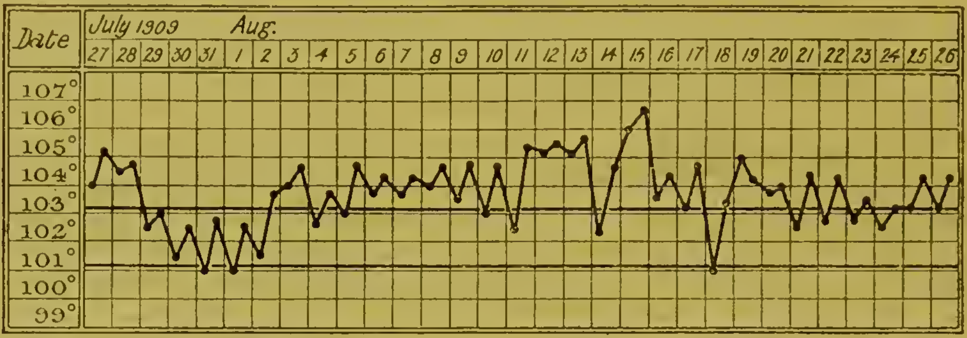


Chart 4.—Experiment 1,386. The Temperature Chart in a Case of Amakebe ending in Recovery.

Experiment 1,635.

To study Amakebe in the Calf.

The following chart represents the course of the temperature:—

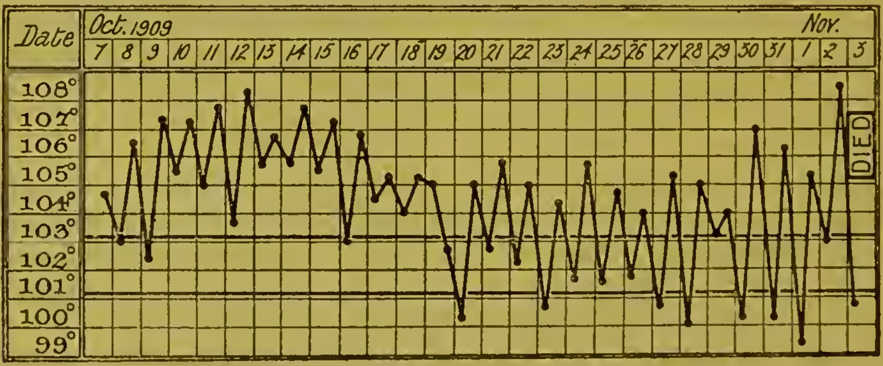


Chart 5.—Experiment 1,635. The Temperature Chart in a Case of Amakebe ending Fatally.

From these foregoing cases and the *post-mortem* examinations, it will be seen that amakebe is an acute disease of calves, and that the main features of the *post-mortem* are signs of intense anæmia, petechiæ of serous membranes, infiltration of jelly-like material into omentum, anterior mediastinum, base of heart, &c., œdema of lungs, swelling and softening of spleen, hæmorrhagic infarcts into lungs, spleen and kidneys, and sometimes ulceration of the mucous membrane of the stomach.

Piroplasms usually Found in the Blood of Uganda Cattle.

When the blood of cattle in Uganda is examined microscopically, two parasites are always to be found, though usually in very small numbers. One of these can readily be recognised as *Piroplasma bigeminum* from its large size and the characteristic appearance of the two pear-shaped bodies (Plate 10, fig. 1). It may, however, also appear as irregularly-shaped, amœboid forms, especially in the spleen (Plate 10, fig. 1). The other parasite is much smaller in size, and is usually seen in the form of a small rod or ring (Plate 10, fig. 2). Both these parasites are inoculable, and appear in the blood of calves without giving rise to any marked disturbance.

Have either of them any connection with amakebe?

The following experiments go to show that they have not:—

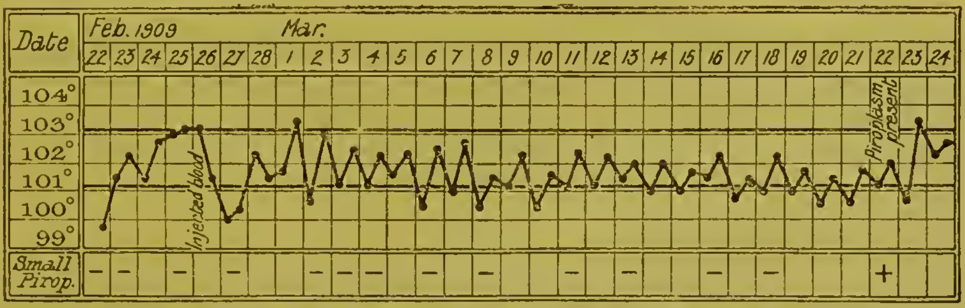
Experiment 556.

To ascertain the effect on a Susceptible Calf of the Injection of Blood containing the Small Rod and Ring-shaped Piroplasm. Will it give rise to Amakebe?

February 22nd, 1909.—This calf was born last night. To-day the mother was cleared of ticks by hand-picking, and then completely smeared with a mixture of paraffin and cyllin, and mother and calf then placed in a tick-free enclosure.

February 26th.—Injected this calf with 5 c.c. blood from calf, Experiment 430, whose blood contains the small rod and ring-shaped piroplasm.

The following chart gives the result:—



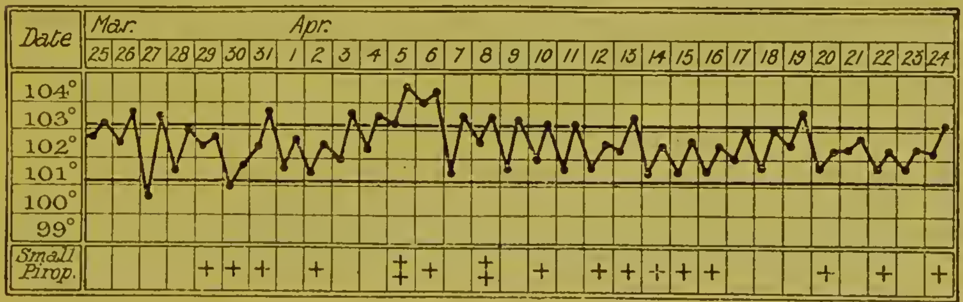


CHART 6.—Experiment 556 represents the Temperature Curve of a Calf which has been Injected with Blood containing the Small Rod and Ring-shaped Piroplasm. The *minus* and *plus* signs show the absence or presence of the small rod and ring-shaped piroplasm in the blood.

Remarks.—Twenty-four days after the injection of the blood containing the small piroplasm, the same rod and ring forms appeared in the blood. The temperature curve hardly shows any response to the invasion of the parasite, and the calf shows no signs of illness. It is evident, then, that the injection of blood containing this small piroplasm gives rise to no symptoms like those seen in amakebe.

In the same way the injection of blood containing *Piroplasma bigeminum* is followed, after some days, by the appearance of this parasite.

Experiment 1,901.

To ascertain if the Injection of Blood containing *Piroplasma bigeminum* will give rise to Symptoms of Amakebe.

August 20th, 1909.—Injected 2 c.c. blood containing *Piroplasma bigéninum* into this calf.

The following chart gives the temperature curve:—

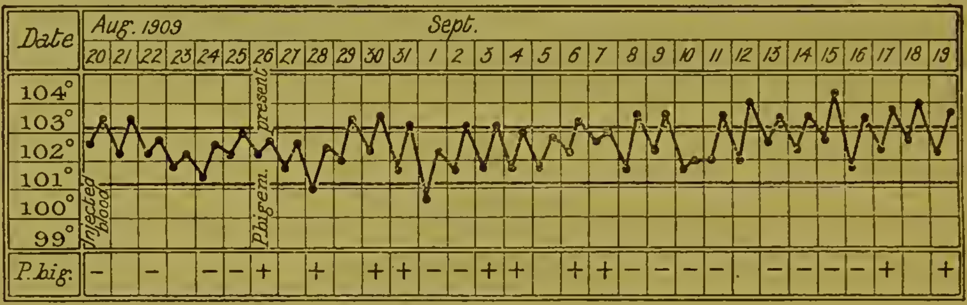


Chart 7.— Experiment 1,901 represents the Temperature Curve of a Calf which has been Injected with Blood containing *Piroplasma bigeminum.* The *plus* and *minus* signs show the presence or absence of *Piroplasma bigeminum* in the blood.

Remarks.—Six days after the injection of blood containing the *Piroplasma bigeminum,* this parasite appeared in the blood. The temperature curve is not affected, nor does the calf appear ill. It may, therefore, be concluded that amakebe is not caused by the injection of blood containing either *Piroplasma bigeminum* or the small rod and ring form.

It is well known that *Piroplasma bigeminum* is carried from affected to susceptible animals by different varieties of the blue tick, as well as other species of ticks. It would seem that the small rod and ring form of piroplasm is carried by the brown tick, as the following two experiments will show.

Experiment 747.

To ascertain if Brown Nymphs which had fed as larvæ on an Animal whose blood contained the Small Rod and Ring Forms, are capable of carrying them to a Susceptible Animal, and if the disease so set up will have the Symptoms of Amakebe.

June 24th, 1909.—This calf, like the others, has been brought up in a tick-free shed. It has been under observation since

May 10 without showing any small rod and ring forms in its blood. To-day a large number of brown nymphs, which had fed as larvæ on an ox whose blood contained the small rod and ring piroplasm were placed on this calf.

The following chart shows the course of the temperature:—

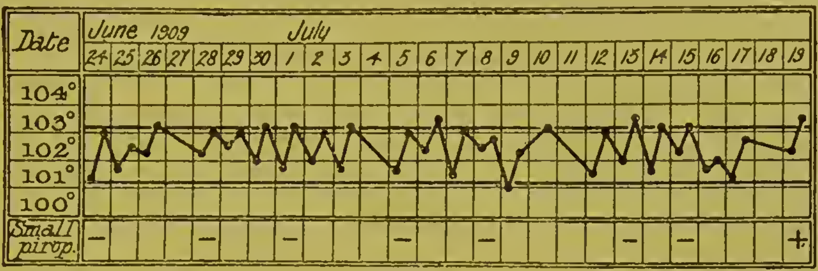


Chart 8.—Experiment 747 represents the Temperature Curve of a Calf upon which Infected Brown Nymphs have been Fed. The *minus* and *plus* signs show the absence or presence of the small rod and ring piroplasm in the blood.

Remarks.—Twenty-five days after the infected brown nymphs were fed on this calf the small rod and ring-shaped piroplasm appeared in the blood. The temperature curve is not affected, and the calf shows no signs of amakebe. It is evident, then, that the small rod and ring-shaped piroplasms transferred to a susceptible calf by means of brown nymphs do not give rise to amakebe.

Experiment 659.

To ascertain if Adult Brown Ticks which had Fed as Nymphs on an Animal whose Blood contained the Small Rod and Ring Forms are capable of carrying them to a Susceptible Animal and setting up the Symptoms of Amakebe.

August 23rd, 1909.—This calf was born on April 4th in a tick-free shed. It has been examined almost daily since that date, and up to the present has shown no parasites of any kind in its blood. To-day a large number of adult brown ticks were placed on this calf.

The following chart shows the course of the temperature:—

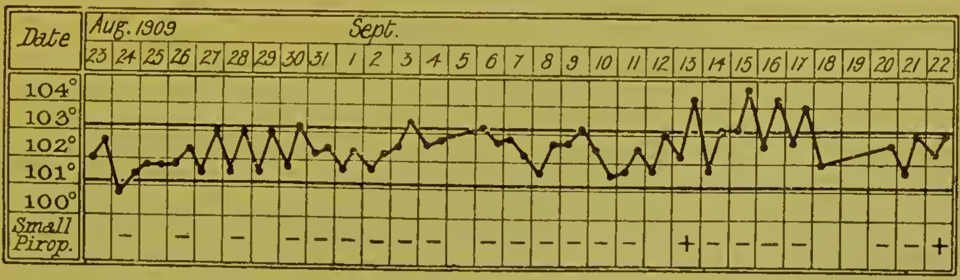


Chart 9.—Experiment 659 represents the Temperature Curve of a Calf upon which Infected Brown Adult Ticks had been Fed. The *minus* and *plus* signs show the absence or presence of the small rod and ring piroplasm in the blood.

Remarks.—Twenty-one days after the infected brown adults had fed on this calf the small piroplasm appeared in the blood.

The temperature curve is only slightly affected, and the calf shows no symptoms of amakebe.

From the foregoing experiments it may be concluded, then, that the appearance of *Piroplasma bigeminum* or of the small rod and ring form of piroplasm in the blood of a susceptible calf, whether introduced by the injection of blood, or in the case of the latter, by the agency of the brown tick, is not accompanied by the symptoms of amakebe. It also is seen from these experiments that the small rod and ring form is inoculable, is carried by the brown tick, and the incubation period is long. This corresponds with the description given by Dr. Theiler, Pretoria, of the piroplasm discovered by him in the Transvaal, and named by him *Piroplasma mutans*.

We may, therefore, consider that the two piroplasms which constantly occur in the blood of Uganda cattle are those known as *Piroplasma bigeminum* and *Piroplasma mutans*, and that neither is the cause of amakebe.

Is Amakebe Inoculable?

It has been shown that blood containing either *Piroplasma bigeminum* or *Piroplasma mutans* if injected into susceptible cattle will give rise to these diseases. Is it equally true that amakebe is inoculable?

The following experiments were carried out to obtain an answer to this question:—

Experiment 1,902.

To ascertain if Blood taken from an Animal suffering from Amakebe and injected into a susceptible Calf will give rise to the Disease.

February 22nd, 1909.—This calf was born last night. Placed in tick-free shed.

February 26th.—Injected with a 5 c.c. blood from calf, Experiment 430, suffering from amakebe.

The following chart shows the result:—

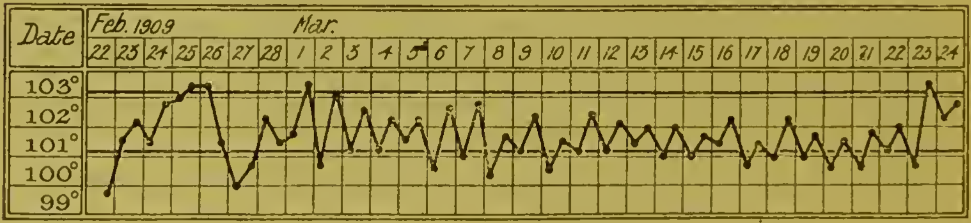


CHART 10.—Experiment 1,902 represents the Temperature Curve of a Calf into which Blood from a Case of Amakebe has been injected.

Remarks.—The temperature curve is not disturbed by the injection of amakebe blood, nor is the calf affected in any way.

Experiment 1,903.

The above experiment repeated.

August 20th, 1909.—Injected 5 c.c. mixture of blood and gland-juice from calf, Experiment 1,387, which is suffering from amakebe.

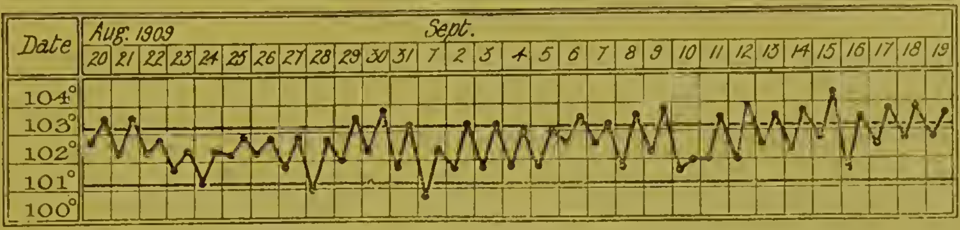


Chart 11.—Experiment 1,903 represents the Temperature Curve of a Calf into which Blood from a Case of Amakebe has been injected.

Remarks.—The result of the injection of amakebe blood is again negative.

Experiment 1,904.

The above experiment again repeated.

August 21st, 1909.—Injected amakebe blood.

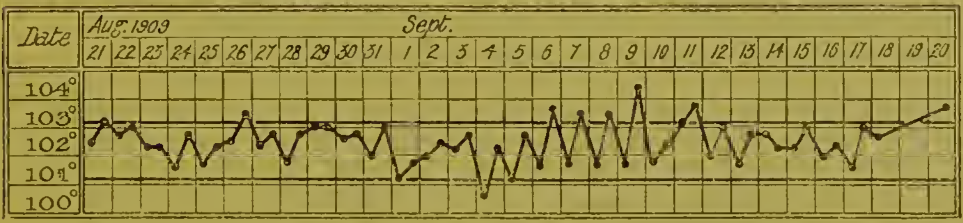


Chart 12.—Experiment 1,904 represents the Temperature Curve of a Calf into which Blood from a Case of Amakebe has been injected.

Remarks.—Result negative.

Experiment 1,905.

September 24th, 1909.—Injected amakebe blood.

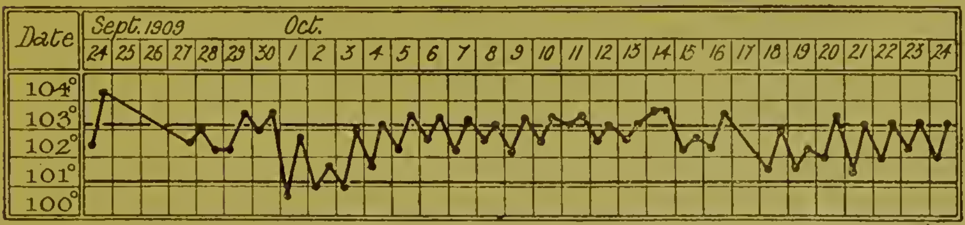


Chart 13.—Experiment 1,905 represents the Temperature Curve of a Calf into which Blood from a Case of Amakebe has been injected.

Remarks.—Result negative.

On three other occasions (Experiments 659, 1,585, and 1,586) was this experiment repeated, and always with a negative result.

It may be concluded, then, that amakebe differs from *Piroplasma bigeminum* and *Piroplasma mutans*, in that it is not inoculable, whereas the latter diseases are.

Result of Exposing Susceptible Calves in a Kraal Contaminated by Amakebe.

Kampala, the native capital of Uganda, has a bad reputation for amakebe. This is probably due to the number of calves stabled in the vicinity. Kampala has a large population of both Europeans and natives, and the milk supply is obtained from private cows kept in the town. The herds of cattle belonging to different individuals are grazed in various parts of the country, but as soon as a cow has calved, she is sent into Kampala to provide milk for her owner. Almost all the calves brought in die of amakebe, which brings about an unhealthy state of things in the cattle kraals where the calves are kept during the day.

The following experiments will show the effect of exposing
To ascertain the Effect of exposing a susceptible Calf in a Kraal

Experiment 1,590.

To ascertain the Effect of exposing a susceptible Calf in Kraal contaminated by Amakebe.

October 11th, 1909.—Sent this calf into Kampala.
October 17th.—Returned from Kampala.

The following chart shows the course of the temperature, and the presence or absence of *Piroplasma bigeminum* or the small rod and ring-formed piroplasma in the blood:—

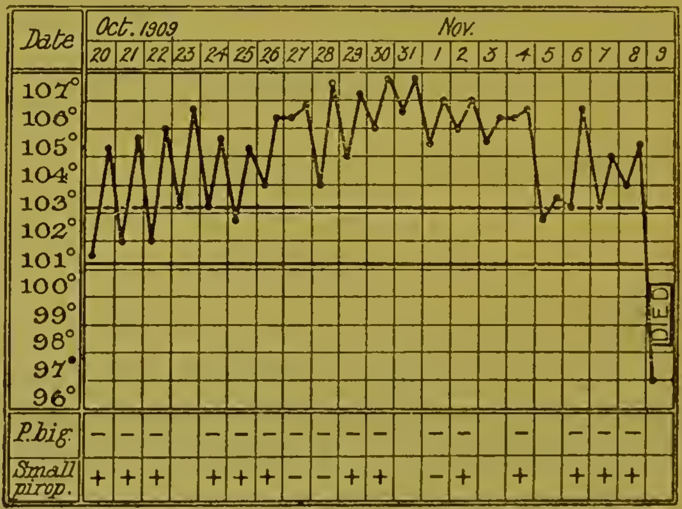


CHART 14.—Experiment 1,590 represents the Temperature Curve of a Calf which has been exposed in a Kraal contaminated by Amakebe. The *plus* and *minus* signs show the presence or absence of *Piroplasma bigeminum* or the small rod and ring piroplasm in the blood.

Remarks.—The result of exposing this calf to a contaminated kraal is an attack of amakebe, characterised by high fever, swollen glands, and death.

Experiment 1,593.

To ascertain the Effect of exposing a susceptible Calf, as in the previous Experiment.

October 11th, 1909.—This calf sent into Kampala.

October 17th.—Returned from Kampala.

The following chart represents the course of the disease, and the presence or absence of *Piroplasma bigeminum* or the small piroplasm in the blood :—

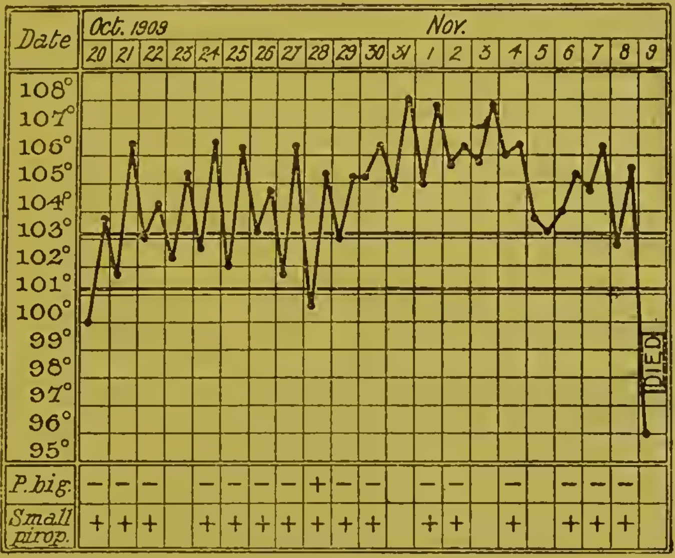


CHART 15.—Experiment 1,593 represents the Temperature Curve of a Calf which has been exposed in a Kraal contaminated by Amakebe. The *plus* and *minus* signs show the presence or absence of *Piroplasma bigeminum* and the small rod and ring piroplasm in the blood.

It is evident, then, that the exposure of susceptible calves for a few days in a kraal where amakebe is common is followed by a serious illness. There is high fever, glandular enlargement, emaciation, and, as a rule, death. This disease has been shown to be caused neither by *Piroplasma bigeminum* nor *Piroplasma mutans*. What, then, is it caused by?

Examination of the Blood in Amakebe.

When the blood of an animal suffering from amakebe is examined, many small piroplasms will be seen (Plate 10, Fig. 3) which appear to be of the same size and shape as *Piroplasma mutans*, and sometimes a few *Piroplasma bigeminum*; otherwise, no new parasite can be said to have come into the blood. This increase in the number of the small piroplasms in the blood of a calf suffering from amakebe, may be explained by saying that the severe illness has led to an excessive multiplication of the *Piroplasma mutans* which was already in the blood. Or, on the other hand, it may be that another species of piroplasm, similar in size and shape to *Piroplasma mutans*, has appeared in the blood, and that the phenomena of amakebe are due to it.

Marginal Points.—Besides the large and small piroplasms, another kind of body is found in the red blood corpuscles, which Theiler has called *marginal points.* In a lecture delivered by him in August, 1909, at Nairobi, in British East Africa, and published in the "Agricultural Journal of British East Africa," October, 1909, he states: "I have recently some to the conclusion that the disease called gall-sickness, and hitherto looked on as a sequel of redwater, is due to the presence of another parasite, which I have called 'Marginal Points' owing to their position in the red blood corpuscles. Gall-sickness is, therefore, a separate and distinct disease." Dr. Theiler considers it proved that this new disease is transmitted by the blue tick. This all shows how complicated and difficult to distinguish are the diseases of cattle. An ox may have *Piroplasma bigeminum*, small rod and ring-shaped piroplasms, marginal points, and one or two species of trypanosome in its blood at the same time. To which parasite have the different phenomena of the disease to be credited?

The two following tables give the blood examination in two cases of amakebe, and illustrate this complexity:—

Experiment 1,387.—Blood Examination in a Case of Amakebe.

Date.	Parasites in Blood.			No. of Red Blood Corpuscles in 1 c. mm. of Blood. Normal 10,000,000.
	Piroplasma bigeminum.	Small Rod and Ring Forms.	Marginal Points.	
1909				
July 28	—	—	+	
,, 29	—	+	—	
,, 30	—	—	+	
,, 31	—	—	+	
August 1	—	—	—	
,, 2	—	—	++	5,420,000
,, 3	—	+	++	5,670,000
,, 4	—	+	—	
,, 5	—	—	+	
,, 6	—	+	++	5,680,000
,, 7	—	+	++	
,, 8	—	+	+	
,, 9	—	+	+	
,, 10	—	—	+	
,, 11	—	—	++	4,420,000
,, 13	—	+	+++	
,, 14	—	+	+++	
,, 16	—	—	+++	4,540,000
,, 19	—	+	+++	4,500,000
,, 23				2,890,000
,, 24	+++	+	+	2,820,000

Table I., Experiment 1,387. The parasites to be found in a case of amakebe. The *plus* and *minus* signs show the presence or absence of these bodies in the blood. The fourth column gives the number of red blood corpuscles in a cubic millimetre. + present, + + numerous, + + + very numerous.

Experiment 1,636.—Blood Examination in a Case of Amakebe.

Date.	Parasites in Blood.			
	Piroplasma bigeminum.	Small Rod and Ring Forms.	Marginal Points.	*Trypanosoma vivax.*
1909.				
September 24	—	—	—	—
" 27	+	+	+	—
" 28	—	+	—	—
" 29	—	—	—	—
" 30	—	—	—	—
October 1	—	—	—	+
" 2	+	+	—	—
" 4	—	—	—	—
" 5	—	+	—	—
" 6	—	++	—	—
" 7	—	+	+	—
" 8	—	+	—	+
" 9	—	+	+	+
" 11	+	+	+	+
" 12	+	+	+	+

Table II., Experiment 1,636. Parasites found in a case of amakebe. The *plus* and *minus* signs show the presence or absence of these bodies in the blood.

Remarks.—From these two tables it will be seen that small piroplasms and marginal points are commonly found in amakebe, and that trypanosomes may also be present.

The marginal points are small, deeply-staining bodies, usually placed near the edge of a red blood corpuscle (Plate 10, Fig. 3). If these bodies really constitute a new and undescribed parasite, the discovery will be one of the greatest interest. Bodies similar in every way to these are found, however, in healthy young rats, goats, calves, &c., so that it is difficult to believe at once in their parasitic nature. Rather would they appear to be cell enclosures, due to rapid changes taking place in the blood, such as take place in young animals or in anæmias. In amakebe they are sometimes very numerous, and it requires no great stretch of the imagination to see in them the youngest stage of the intra-corpuscular parasite, which from being round becomes wedge-shaped, oval, or circular, and rod-shaped. It may be that both these views are true—that some of the so-called marginal points are remains of chromatin from some previous nuclear structure, and that others are the earliest stages of an intra-corpuscular parasite. More work is required before any definite conclusion can be arrived at.

Koch's Granules or Blue Bodies.—Another body which may sometimes, though rarely, be seen in the blood of amakebe calves, is one similar to that first described by Koch, and known as Koch's Granules or Blue Bodies. They are found principally in the spleen, lymphatic glands and liver, where they may be quite numerous. Stained by Giemsa the body appears as a blue-coloured cell, filled with coarse chromatin granules (Plate 10, Fig. 5).

The following table gives cases of amakebe in which these bodies were found:—

Experiment.	Date.	Spleen.	Liver.	Lymphatic Glands.	Kidney.	Lung.	Blood.
	1909.						
415	May 10 ...	+					
1392	July 24 ...	+					
1593	Nov. 10 ...	+					
1633	Oct. 5 ...	+++	+++	+++			
1634	,, 18 ...	++	++	+	+	+	
1635	,, 15 ...						+
1636	,, 12 ...	+	+	+	+	+	
1637	,, 6 ...	+	+	+	+	+	
1638	,, 6 ...	++	+	++	+	+	+
1833	—	+	+		+		
1888	Nov. 5 ...	++					
1891	,, 8 ...	+					
1908	,, 14 ...	++					

Table III., showing the presence of blue bodies in cases of amakebe. + present, + + numerous, + + + very numerous, — absent.

Diagnosis of Amakebe.

What, then, is amakebe? In the opinion of the Commission it is the disease of cattle discovered by Koch, and named by him East Coast Fever. The chief ground for this opinion are, the symptoms during life, the appearances after death, the occurrence of a small piroplasm in the blood indistinguishable from *Piroplasma parvum*, and lastly and chiefly, the presence of the blue bodies in the spleen and other organs. These bodies have never been known to occur in any other disease, and the diagnosis of East Coast fever is made in South Africa if such bodies are found in spleen smears.

Conclusions.

1. The blood of cattle in Uganda almost always contains *Piroplasma bigeminum* and *Piroplasma mutans*, and the cattle are therefore immune to these two diseases.

2. The disease of calves called amakebe is East Coast fever, so that very many of the cattle in Uganda are almost immune to this disease.

3. Owing to the nature of East Coast fever, inasmuch as animals recovered from the disease are no longer infective, some calves may escape attack of amakebe, and so remain susceptible.

4. Thus the calves of the Sesse Islands escape amakebe, and when as grown-up cattle they are transferred to the mainland, they mostly die of East Coast fever.

5. The carriers of East Coast fever—*Rhipicephalus appendiculatus*, or brown tick; *Rhipicephalus evertsi*, or red-legged tick; and *Rhipicephalus simus*—are all common in Uganda.

DESCRIPTION OF PLATE 10.

Fig. 1.—The two upper corpuscles show the characteristic pear-shaped forms of *Piroplasma bigeminum* as they appear in the blood. The lower amœboid forms are drawn from a preparation of spleen. Stained Giemsa. × 2000.

Fig. 2.—*Piroplasma mutans* in the blood. Stained Giemsa. × 2000.

Fig. 3.—The small rod and ring-shaped piroplasm, as seen in the blood of a case of amakebe. Among them are the deeply-stained bodies known as marginal points. Stained Leishman. × 2000.

Fig. 4.—Red blood corpuscles containing piroplasms from the spleen of a case of amakebe. Stained Giemsa. × 2000.

Fig. 5.—Koch's granules or blue bodies from the spleen of a case of amakebe. Stained Giemsa. × 2000.

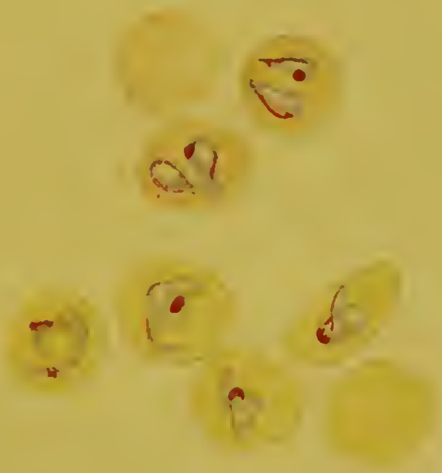


Fig 1.

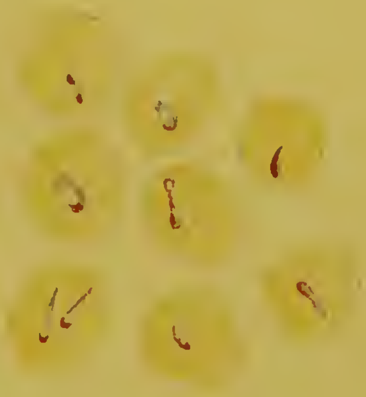


Fig. 2.

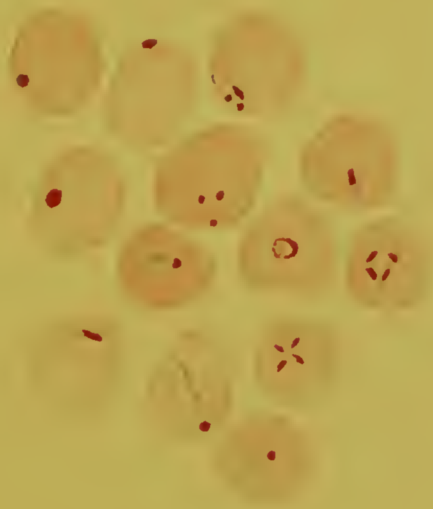


Fig. 3.

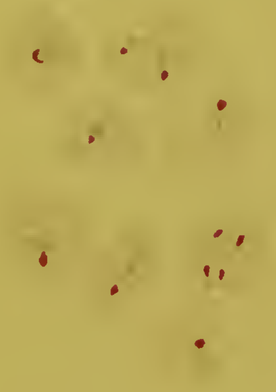


Fig. 4.

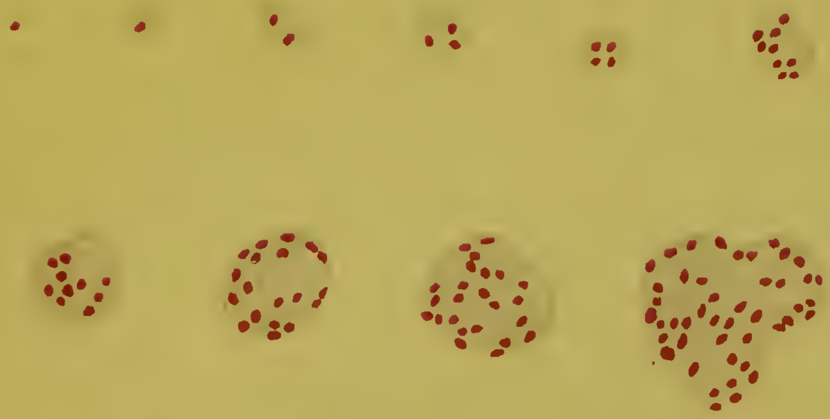


Fig 5.

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